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MECHANISMS UNDERLYING THE EFFECTS OF PHOTOPERIOD ON THE ACCESSORY SEX GLANDS OF ADULT MALE SYRIAN GOLDEN HAMSTER

(Mesocricetus auratus)

BY

JEAN G. TOMA

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ANATOMY AND CELL BIOLOGY

EDMONTON, ALBERTA
(SPRING, 1988)

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ISBN 0-315-42968-2

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TITLE OF THESIS: MECHANISMS UNDERLYING THE EFFECTS OF PHOTOPERIOD ON THE ACCESSORY SEX GLANDS OF ADULT MALE SYRIAN GOLDEN HAMSTER (*Mesocricetus auratus*).

DEGREE: DOCTOR OF PHILOSOPHY.

YEAR THIS DEGREE GRANTED: SPRING, 1988.

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **MECHANISMS UNDERLYING THE EFFECTS OF PHOTOPERIOD ON THE ACCESSORY SEX GLANDS OF ADULT MALE SYRIAN GOLDEN HAMSTER (*Mesocricetus auratus*)** submitted by **JEAN G. TOMA** in partial fulfilment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in Anatomy and Cell Biology.

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Date: 1 March, 1988

ABSTRACT

In the present study young adult male Syrian hamsters were used in experiments which were designed to determine the effects of photoperiods on the weights and the fine structure and DNA and protein content of the major accessory sex glands, and to define the effects of photoperiods, combined with other endocrine manipulations on these glands in order to try to sort out direct from indirect effects. The results of these studies indicated: 1) exposure of intact hamsters to short daily photoperiods for ten weeks resulted in a significant reduction in the weights of all accessory sex glands, relative to those maintained in long days; among these glands the ventral lobes of the prostate showed the least reduction; 2) short-day exposure resulted in marked changes in the fine structure of the secretory cells of these glands, suggesting suppression of cytological secretory activity; 3) short-day exposure resulted in a significant reduction in the total protein content of the ventral and dorsal lobes of the prostate, and a marked reduction in the total DNA content of the dorsal prostate; 4) the reduction in weights, DNA and protein content of the accessory sex glands was more rapid with castration than with exposure to short days; 5) short-day exposure resulted in a consistent reduction in the circulating levels of testosterone and prolactin; 6) the presence of excess prolactin in animals maintained in short days did not prevent most of the atrophic responses of the accessory sex glands or the suppression of

circulating testosterone levels; 7) the presence of an adequate supply of testosterone in castrated hamsters prevented the effects of short days on the weights, DNA and protein contents of the accessory sex glands; and 8) the suppressive effects of short days on the accessory sex glands and on circulating reproductive hormones are pineal mediated phenomena as removal of the pineal gland negated the effects of short days. These results suggest that the effects of short days on the accessory sex glands are primarily due to a decrease in circulating testosterone levels, and are likely mediated indirectly via the effects of the pineal secretion on the neuroendocrine-gonadal axis.

ACKNOWLEDGMENTS

My sincere gratitude is expressed to my supervisor Dr. G.R. Buzzell and to supervisory committee members Dr. N.C. Milos and Dr. L.C.H. Wang for their valuable advice and support throughout the course of this study.

I would also like to express my deep appreciation to Dr. R.J. Reiter, Dr. D.E. Blask and Dr. G. Bourne for their contribution with the hormone radioimmunoassays.

My deepest appreciation is also extended to Dr. J.F. Henderson and Dr. A.R. Morgan who provided both valuable advice and laboratory facilities for DNA and protein assays.

Special thanks to Mrs. J. Haskins and Mr. G. Morrison for their technical assistance.

I would also like to extend special thanks to the faculty and staff as well as my colleagues in the Department of Anatomy and Cell Biology at the University of Alberta. Their valuable assistance and cooperation in various capacities is sincerely appreciated.

I am also grateful to my sister Sarah Toma for her concern, support and encouragement over the years.

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LIST OF ABBREVIATIONS

AG - ampullary gland
BC - basal cell
BL - basal lamina
CAST. - castrated
CG - coagulating gland
DB - dense body
DP - dorsal lobes of the prostate
E - epithelium
Fb - fibroblast
FSH - follicle stimulating hormone
G - Golgi complex
HIOMT - Hydroxyindole-O-methyltransferase
IT - interacinar tissue
L - lumen of acinus
LD - Long-day photoperiods
LH - luteinizing hormone
LP - lamina propria
Ly - lysosome
M - mitochondria
MV - microvilli
N - nucleus
NAT - N-acetyltransferase
NE - Norepinephrine
Nu - nucleolus
PINX - pinealectomy
PRL - prolactin
RER - rough endoplasmic reticulum
SD - Short-day photoperiods
SCG - Superior cervical ganglion
SCN - Suprachiasmatic nucleus
SG - secretory granules
SM - smooth muscle forming muscular stroma.
SV - seminal vesicles
T - testosterone
V - vacuole
VP - ventral lobes of the prostate

I- INTRODUCTION

Accessory Sex Glands

Gross Anatomy and Histology:

The accessory sex glands can be divided into two major groups according to their developmental origin. The glands originating from the mesonephric or Wolffian duct (vas deferens) are the ampullary glands and seminal vesicles. The glands that derive from the urogenital sinus include the prostate, bulbo-urethral and urethral glands.

There are marked differences between mammalian orders with respect to the accessory sex glands present. For example, carnivores such as the dog and cat lack seminal vesicles and cetaceans lack seminal vesicles, ampullary glands and bulbourethral glands. The only gland found in all orders of mammals is the prostate (Price and Williams-Ashman, 1961).

The accessory sex glands also vary among different species of mammals, both morphologically and in the biochemical composition of their secretions. The gross structure of the prostate varies from a disseminate or diffuse type, in which the glandular units remain in the urethral stroma, to one which is more discrete, in which the glandular units form an external body that is sometimes lobed outside the urethral muscle. A disseminate type of prostate is found in the marsupials (Rodger and Hughes, 1973). The prostate in adult man and dog is a solid compact organ. Rodents have a lobular prostate in which lobes are present which are distinctly separate (Price, 1963). In rodents such as the rat, the prostate is composed of discrete paired ventral lobes, dorsolateral lobes, and the paired anterior or cranial lobes which are also termed the coagulating glands (Price, 1963; Jesik et al., 1982).

The accessory sex glands of the Syrian golden hamster (*Mesocricetus auratus*) generally resemble those of the rat. Figure I-1 illustrates the major accessory sex glands of the adult male hamster. The largest and most conspicuous glands are the seminal vesicles. Each of these saccular glands empties by a single opening into the vas deferens just before the vas opens into the urethra. This region of the vas is expanded to form the ampullary gland. The coagulating glands lie nestled in the lesser curvature of the seminal vesicles, with which they share a common peritoneal sheath. Each lobe of the coagulating glands empties via a single duct into the urethra near the opening of the vasa deferentia. The ventral lobes of the prostate consist of a pair of small clearly separate lobes, each of which is embedded in fat and lies adjacent to the ventrolateral surface of the urethra and the neck of the bladder. The lateral lobes are not present in the hamster. The dorsal lobes of the prostate consist of two large nearly fused lobes that lie on the dorsal surface of the urethra. The ventral and dorsal lobes empty their secretions into the urethra through a number of ducts.

In all mammalian accessory sex glands, the lumina of the glandular units are lined with a simple columnar secretory epithelium containing occasional basal cells, which rests on a basal lamina and

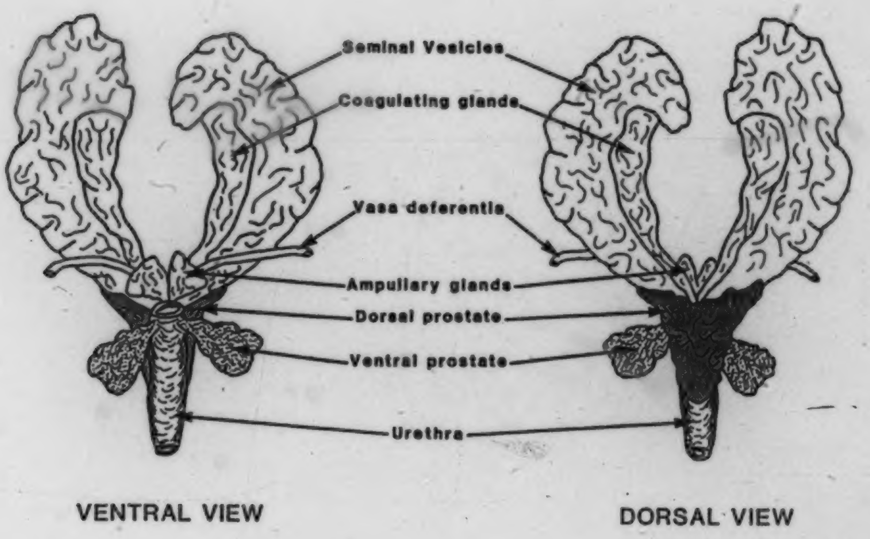
Seminal Vesicles
Coagulating glands
Vasa deferentia
Ampullary glands
Dorsal prostate
Ventral prostate
Urethra
VENTRAL VIEW
DORSAL VIEW

Fig. I-1. Diagram to illustrate the major accessory sex glands of the male Syrian hamster, from the ventral and dorsal view.

a thin layer of lamina propria. This is, in turn, supported by a fibromuscular stroma. The mucosa (epithelium and lamina propria) within the secretory units is usually folded and each fold has a thin core of lamina propria. Each individual lobe is covered by a capsule mainly made of connective tissue (Price and Williams-Ashman, 1961; Brandes, 1966; Salander et al., 1981; Jesik et al., 1982). Although all accessory sex glands have histological features in common, ultrastructural analysis of the epithelium of the seminal vesicles and different lobes of the prostate of rat, mouse and guinea pig has revealed some clear differences in the fine structure of these organs (Brandes, 1966; Veneziale et al., 1974; Dahl et al., 1973; Wong and Tse, 1981).

Biochemical and Functional Properties:

Despite numerous reports on the biochemical composition of whole semen, seminal plasma, and split ejaculates from various species (Mann and Lutwak-Mann, 1981; Polakoski and Kopta, 1982), relatively little has been learned about the composition of the secretions from the individual accessory sex glands. Additionally, the functional significance of many of these secretions remains obscure. Part of the reason for this is the tremendous variability in accessory sex glands among species, both morphologically and in the biochemical composition of their secretions.

The secretion of the accessory sex glands is a highly complex mixture of proteins and nutrients which constitute the seminal plasma. Fructose and citric acid, characteristic constituents of the seminal plasma, are secreted by the accessory sex glands and the sites of their formation vary from one species to another. For instance, in most mammals, including man and the guinea pig, fructose in semen originates mainly from the seminal vesicles and ampullary glands (Huggins and Johnson, 1933; Mann; 1946; Davies and Mann, 1947; Mann and Lutwak-Mann, 1951; Ortiz et al., 1956), whereas in the rat, the main fructose producing organs are the coagulating glands and the lateral lobes of the prostate (Mann, 1946; Humphrey and Mann, 1948; Harding et al., 1963; Gerhardt et al., 1983). Furthermore, in the rat, citric acid is produced by the seminal vesicles and the ventral prostate, whereas in man, it is produced only by the prostate gland (Harding et al, 1963; Mann and Lutwak-Mann, 1981; Gerhardt et al., 1983). Fructose in seminal plasma serves as a source of energy for spermatozoa, whereas citric acid is connected with coagulation and liquefaction of semen (Huggins and Neal, 1942; Mann, 1964). Another possible function of citric acid is that, in combination with sodium and potassium ions, it may play a part in maintaining the osmotic equilibrium of semen (Mann, 1964).

Numerous proteins and enzymes have been demonstrated in the secretion of the seminal vesicles and prostate. The ventral prostate of the rat secretes a large amount of a glycoprotein which plays a major role in the nonspecific steroid-binding of prostatic cytosol (Heyns et al., 1978; Heyns and de Moor, 1977). The dorsal prostate and the coagulating glands have also been found to synthesize nearly identical secretory proteins in response to androgen (Wilson and French, 1980; Bartlett et al., 1984). These proteins have been referred to as dorsal protein I (DP I) and dorsal protein II (DP II).

DP II has been shown to be the major component of the coagulating gland fluid and the sperm-carrying unclotted portion of rat seminal fluid (Bartlett et al., 1984). Nonetheless, the functional significance of these proteins in reproductive biology of the rat is unknown.

Accessory sex glands produce proteins necessary for coagulation of semen. The seminal vesicles of rodents and man produce the protein components of the coagulum (Huggins and Neal, 1942; Manyai, 1964; Manyai et al., 1965; Hart, 1970; Koren et al, 1975; Higgins et al., 1976; Tauber and Zaneveld, 1976; Bradshaw and Wolfe, 1977; Mann and Lurwak-Mann, 1981). In rodents, the seminal vesicles produce the enzyme procoagulase and the coagulating gland produces other enzymes, such as transmidase and vesiculase. These enzymes are important for the coagulation of semen and formation of the copulatory (vaginal) plug in rodents (Gotterer et al., 1955; Gotterer and Williams-Ashman, 1957; Zorgniotti and Brendler, 1958; Speyer, 1959; Hart, 1970; Joshi et al., 1972; Beil and Hart, 1973; Williams-Ashman et al., 1977). The human prostate also produces several strong proteolytic enzymes that are important for the liquefaction of semen (Huggins and Neal, 1942; Ying et al., 1956; Nijs et al., 1971; 1973; Brassinni et al., 1976; Tauber and Zaneveld, 1976).

One of the known phosphatases found in the secretions of the male accessory sex glands of various animals is acid phosphatase. High levels of this enzyme are present in the prostate glands of dog, monkey and man, and in the seminal vesicles of the guinea pig and rat (Bern and Levy, 1952; Price and Williams-Ashman, 1961; Bell and Lake, 1962; Gyorkey, 1964; Seligman et al., 1975; Gerhardt et al., 1983). Levels of acid phosphatase are relatively low in the prostates of the rabbit, rat and bull (Bern and Levy, 1952; Price and Williams-Ashman, 1961; Bell and Lake, 1962; Seligman et al., 1975; Gerhardt et al., 1983). In man, acid phosphatase is secreted by normal, neoplastic and hyperplastic prostatic epithelial cells (Kirchheim et al., 1964; Dounis, 1977; Lin et al., 1980; Bruce et al., 1981). Human prostatic acid phosphatase hydrolyzes a number of phosphate monoesters and catalyzes the transfer of phosphoryl groups from various substrates to acceptors such as aliphatic alcohols (London and Hudson, 1955; Jeffree, 1957; Nigam and Fishman, 1959; Nigam et al., 1959; Ostrowski and Bernard, 1973; Ostrowski, 1980). Serum acid phosphatase in man has been used as an aid in the diagnosis of prostate cancer (Cooper and Foti, 1974; Bruce et al., 1981). Alkaline phosphatase is also present in the prostatic secretion of man and bull, and in the prostate and seminal vesicles of mouse, rat and guinea pig (Litosova, et al., 1969; Kimura and Ichihara, 1980; Mann and Lutwak-Mann, 1981).

Another characteristic feature of the prostatic secretion is the high concentration of zinc (Byar, 1974; Habib, 1978). Gunn et al. (1955) demonstrated that the dorsolateral prostate of rat has an ability to concentrate zinc to levels that are 15-25 times higher than any other organ. The lateral portion of the rat dorsolateral prostate specifically expresses this avidity for zinc (Gunn and Gould, 1956a; Rixon and Whitfield, 1959; Byar, 1974). High concentrations of zinc have also been observed in the coagulating glands of rats (Gerhardt et al. 1983). The ability to sequester and maintain a high zinc concentration in rat dorsolateral prostatic tissue is dependent upon the presence of testosterone (Gunn and Gould, 1956b; Muntzing et al., 1977) and is influenced by pituitary hormones such as luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin (Rosoff and Martin, 1968; Moger and Geschwind, 1972; Itaya et al., 1984). Moger and Geschwind (1972) demonstrated that the *in vitro* uptake of zinc into rat prostate was augmented by administration of prolactin, whereas a study by Leake et al. (1984) demonstrated that zinc enhanced the *in vitro* uptake of prolactin into human benign prostatic hypertrophic tissue and this was partly due to an increase in the non-specific binding properties of prolactin. Nonetheless, the precise physiological role that zinc plays in the prostate gland remains unknown.

The secretions of the accessory sex glands also contain lipids, phospholipids, prostaglandins, choline derivatives, polyamines such as spermine and spermidine, and several electrolytes which are important for the ionic equilibrium of the seminal fluid (Mann and Lutwak-Mann, 1981; Beyler and Zaneveld, 1982).

Several studies have been conducted to determine the functional significance of the accessory sex glands, and to test whether all or some of these glands are necessary for the preservation of fertility and fecundity in rodents. It has been reported that removal of the seminal vesicles of the mouse (Pang et al., 1979; Peitz and Olds-Clarke, 1986), guinea pig (Lawlah, 1930) and rat (Blandau, 1945, Queen et al., 1981) prevented the formation of the copulatory plug and impaired the fertility of these animals. In the hamster, on the other hand, Chow et al. (1986) demonstrated that only the ventral prostate appeared to be essential for fertility. In mice, removal of the ventral and dorsal prostate has also been reported to significantly reduce fertility (Pang et al., 1979). Thus it appears that amongst the rodents, the various accessory sex glands differ between species in their contributions towards fertility.

Hormonal Control:

The male accessory sex glands are dependent on androgens for their normal structure and function. Removal of the androgenic stimulus, such as by castration, hypophysectomy, and other antiandrogenic procedures, induces structural involution and an associated decrease or loss of functional activities (Brandes, 1966; Dahl and Kjaerheim, 1973; Dahl and Tveter, 1973; Cavazos, 1975).

In the normal male, the major circulating androgen is testosterone. More than 95% of plasma testosterone is produced by the testes and a minor portion is produced by the peripheral conversion of other steroids such as androstenedione (Coffey and Isaacs, 1981). Testosterone is produced and secreted chiefly by the interstitial cells or Leydig cells of the testes (Christensen and Mason, 1965; Hall et al., 1969; Eik-Nes, 1971; Podesta et al., 1978; Dufau et al., 1984). In addition to testosterone, there are a number of other androgens that are also produced by the testes including androstanediol, androstenedione and dehydroepiandrosterone (Eik-Nes and Kekre, 1963; Hall et al., 1964; Ibayashi et al., 1965; Yamaji et al., 1968; Laatikainen et al., 1971; Eik-Nes, 1975). However, these androgens are relatively weak and their concentrations are much lower than those of testosterone.

The androgens are, in part, transported in the blood by binding to specific plasma proteins. About 98% of the testosterone circulating in the plasma is bound to protein [testosterone-binding protein (albumin, specific globulins and other proteins)] (Mercier-Bodard et al., 1970; Spaziani, 1975; Sandberg, 1980; Coffey and Issacs, 1981). Therefore, under normal circumstances, only a small fraction of circulating testosterone is in the free and active form. Protein-bound steroid is not physiologically active, and is not available to intracellular receptors, except when it dissociates and diffuses through the plasma membrane of the target cell. Blood testosterone levels in man and in other animals are significantly influenced by age, schedule of blood sampling, luteinizing hormone (LH) available to the testes around the blood sampling, stress, nutrition, season, and photoperiodic and other environmental factors (Grewal et al., 1971; Ghanadian et al., 1975; Leathem, 1975; Rigaudiere et al., 1976; Bronson and Desjardins, 1977; Chan et al., 1977; Gray, 1978; Keating and Tcholakian, 1979; Collu et al., 1984; Turek et al., 1984).

Testosterone, like other steroid hormones, is known to act by stimulating protein synthesis within its target tissues. In the prostate gland, seminal vesicles and several other tissues that are primary targets for testosterone, unbound testosterone is taken up by target cells by passive diffusion or possibly by a specific transport system (Giorgi et al., 1973). In these target cells, testosterone is converted to 5α-dihydrotestosterone (5α-DHT) and other 5α-metabolites by the action of the enzyme 5α-reductase (Anderson and Liao, 1968; Bruchovsky and Wilson, 1968; Bashirelahi and Villee, 1970; Wilson and Gloyna, 1970; Liao et al., 1974). In the cytoplasm, 5α-DHT binds to specific, high affinity receptor molecules (Fang et al., 1969; Mainwaring, 1975; Foekens et al, 1985). The cytoplasmic receptors bind 5α-DHT in preference to testosterone (Fang et al., 1969; Mainwaring, 1969). The steroid-receptor complex then migrates from the cytoplasm to the nucleus and binds to specific acceptor sites. The exact nature of the nuclear acceptor is unknown. DHT-receptor complex interacts with many nuclear components, such as the nuclear membrane (Lefebvre and Novosad, 1980), non-histone proteins (Tymoczko and Liao, 1971; Klyzsejko-Stefanowicz et al., 1976; Mainwaring et al., 1976; Wang, 1978), RNA and ribonucleoprotein particles (Liao et al., 1973, 1980; Mulder et al., 1984), nuclear matrix (Barrack, 1983; Barrack and Coffey, 1980), and DNA (Yamamoto and Alberts, 1972; Mainwaring and Irving, 1973; Rennie, 1979; Buttyan et al., 1983; Foekens et al., 1985). It is generally assumed that this binding or interaction can result in alterations in the expression of specific genes leading to the formation of mRNA and the result is increased formation of specific protein molecules that are important for development, growth and maintenance of these target cells (Isotalo and Santti, 1975; Spaziani, 1975; Mainwaring et al., 1974, 1976; Yamamoto and Alberts, 1976; Judge and Jaffe, 1982; King, 1982; Tenniswood et al., 1982; Scheidereit et al., 1986).

Several recent studies controvert the classical cytoplasmic localization of steroid receptors, which, upon binding of the hormone would translocate into the nuclear compartment (King and Greene, 1984; McClellan et al., 1984; Press and Greene, 1984; Molinari et al., 1985; Perrot-Applanat et al., 1985; Welshons et al., 1985; Baulieu, 1986; Gasc and Baulieu, 1986). The new data demonstrate that steroid receptors are located in the nuclear compartment under all hormonal conditions, thus rendering the hypothetical nuclear translocation unnecessary.

Testicular function is controlled by pituitary follicle-stimulating hormone (FSH), luteinizing hormone (LH) and prolactin. The hypothalamus produces luteinizing hormone-releasing hormone (LH-RH) which stimulates the release of both LH and FSH from the anterior pituitary gland (Schally et al., 1972, 1973; Wise et al., 1979). The Leydig cells of the testes are stimulated by gonadotropins (LH and FSH), primarily by LH, to synthesize testosterone from acetate and cholesterol (Chatterton, 1982; Cheesman, 1982; Ewing et al., 1983; Dufau et al., 1984; Lieberman et al., 1984). Increased testosterone levels cause feedback inhibition of LH secretion by acting on the hypothalamus and inhibiting the production of LH-RH. Testosterone may act also on the pituitary gland to reduce sensitivity of gonadotrophs to LH-RH (Nansel and Trent, 1979; Nansel et al., 1979).

Although the major site of FSH action is the Sertoli cells (which are important for spermatogenesis), it has also been reported that FSH has a role in stimulating testosterone production *in vivo* and *in vitro* in adult mice (Dalterio et al., 1986) and enhances the number of Leydig cell LH receptors and thus potentiates the effects of LH on testosterone production (Bartke et al., 1978; Nazian and Mahesh, 1981). It has also been suggested that FSH may stimulate cyclic nucleotide production (Heindel et al., 1978) or influence the hydroxysteroid dehydrogenase/ketosteroid isomerase systems leading to increased substrate availability for testosterone biosynthesis (Shaw et al., 1979).

Prolactin has also been implicated as a factor in the growth and maintenance of the male accessory sex organs. In rodents, prolactin has been reported to have positive effects on weight (Grayhack, 1963; Bartke and Lloyd, 1970; Negro-Vilar, et al., 1977), nucleic acid levels (Thomas and Manandhar, 1975; Prins and Lee, 1983), cell proliferation (McKeehan, et al., 1984), protein synthesis (Thomas and Manandhar, 1975), zinc uptake (Moger and Geschwind, 1972), citric acid concentration (Grayhack, 1963; Grayhack and Lebowitz, 1967; Walvoord et al., 1976; Slaunwhite and Sharma, 1977) and acid phosphatase activity (Ghosh et al., 1983).

Prolactin may directly or indirectly influence the growth and function of accessory sex glands in different species of mammals. The magnitude of this response depends on the strain, tissue and details of experimental design. Administration of prolactin alone to castrated male rats and mice causes a small but detectable increase in the weights of the accessory sex glands. This effect is not mediated through pituitary or adrenal secretions, suggesting a direct action of prolactin on the accessory sex glands (Bartke and Lloyd, 1970; Negro-Vilar et al., 1977). Biochemical and histochemical studies have demonstrated that specific prolactin binding sites are present in the prostate gland and seminal vesicles of the rat (Aragona and Friesen, 1975; Barkey et al. 1977, 1979; Witorsch, 1978, 1981; Thompson et al., 1982).

In addition to direct effects of prolactin on the accessory sex glands, this hormone may act indirectly on these organs by increasing the biosynthesis and secretion of testosterone from the testes (Hafiez et al., 1972; Bartke et al., 1978) and by synergizing with testosterone and dihydrotestosterone within the accessory sex glands (Walvoord et al., 1976; Thompson and Heidger, 1978; Negro-Vilar, 1980; Prins and Lee, 1982).

Although prolactin has been shown to have stimulatory effects on the accessory sex glands of several species of rodents, there are many conflicting reports concerning the degree of responsiveness of the seminal vesicles and different lobes of the prostate to circulating prolactin (Chase et al., 1957; Bartke, 1967; Bartke and Lloyd, 1970; Yamanaka et al., 1975; Witorsch, 1978; Holland and Lee, 1980; Keenan et al., 1981). The magnitude of the response appears to be dependent on strain, age, tissue and details of experimental design. Nonetheless, it may be concluded that prolactin affects the growth and function of the accessory sex glands in at least three different ways: 1) it may increase the output of testosterone from the testes, 2) it may act synergistically with testosterone by potentiating its effects, and 3) it may promote growth and function by acting directly on the accessory sex glands.

Although physiological levels of prolactin have stimulatory effects on the male reproductive tract in several mammalian species, excessive prolactin release in men with pituitary tumors usually results in a decline in sexual and reproductive functions (Thorner and Besser, 1978). In male mice and rats, hyperprolactinaemia suppresses copulatory behaviour (Svare et al., 1979), and in male rats suppresses the post-castration increase in the plasma concentrations of both LH and FSH (Brar et al., 1985). Thus, it appears that prolactin is normally involved in the control of male reproductive function, and it can exert both stimulatory and inhibitory effects on the male reproductive system.

Besides testosterone and prolactin, direct and indirect effects of estrogen, and estrogen interactions with testosterone on male accessory sex glands have been studied from a variety of aspects. Estrogen administration can induce either regression or growth of the accessory sex glands, depending on the hormonal status of the host.

Although in intact animals, estradiol administration produces atrophy of the accessory sex glands (Price and Williams-Ashman, 1961; Tesar and Scott, 1964), a number of studies have reported that estrogens are able to partially maintain the weights of the seminal vesicles and various lobes of the prostate in castrated male rats (Tisell, 1971, 1972; Tisell et al, 1976; Robinette et al., 1978; Belis et al., 1983), and partially maintain the weights of the seminal vesicles and lateral lobes of the prostate in castrated adrenalectomized male rats (Tisell, 1971). Estrogen given to castrated male rats has been shown to produce stimulatory growth effects including fibromuscular hypertrophy (Thompson et al., 1979) and/or epithelial hyperplasia (Tisell et al., 1976; Salander and Tisell, 1976). A study by Lee et al. (1981) indicated that the stimulatory effects of estrogen in castrated rats were mediated through the pituitary. In the absence of testosterone and a functional pituitary gland, estradiol was unable to stimulate prostatic growth; rather, it delayed the rate of castration-induced regression in all lobes of the prostate gland.

Estrogen may act indirectly on the accessory sex glands by reducing the testicular output of androgens. The treatment of intact male rats with estrogen leads to a decrease in circulating LH and testosterone levels (Swerdloff and Odell, 1968; Gay and Dever, 1971; Kalra et al., 1973; De Jong et al., 1975; Nishihara and Takahashi, 1983). The decrease in testosterone concentration has been attributed to the reduction of LH secretion, resulting from negative feedback actions of estrogen on the hypothalamus and/or the pituitary gland.

In addition to indirect effects of estrogen, this steroid may act directly on Leydig cells, as these cells have been shown to contain estrogen receptors (van Beurden-Lamers et al., 1974). *In vitro* studies have indicated that estradiol inhibits the activity of testicular steroidogenic enzymes, suggesting direct effects of estrogen on testosterone production (Sholiton et al., 1975; Brinkmann et al., 1980).

Thus, there are several hormones involved in control of the growth and function of the accessory sex glands, and these hormones may act directly or indirectly on these organs. The secretion of these hormones is governed by intrinsic and extrinsic regulatory factors. Intrinsic factors include receptors, down-regulation of receptors and feedback effects of other hormones. The extrinsic factors involve stress, special sensory input such as tactile stimuli of copulation or of suckling, olfaction, and visual input (Gorski, 1979; Collu et al., 1984). With respect to visual input, it is necessary to consider the importance of photoperiods, which play an important role in the reproductive physiology of several species of mammals. It is now well established that the effects of photoperiod on the reproductive system are primarily mediated by the pineal gland (Reiter, 1980b).

The Pineal Gland

It is now well established that the pineal gland (epiphysis cerebri) is involved in the control of reproductive processes of certain mammals, possibly even in humans. The pineal is an essential component of the neuroendocrine system regulating circannual rhythms of endocrine activity. However, the exact mechanisms by which the pineal affects reproductive processes are still unclear. It is generally believed that the pineal secretes a hormone which modulate the reproductive function. A vast amount of experimental data indicates that melatonin, an indole, is the pineal hormone which mediates many of the effects of the pineal gland on the reproductive system, and has both progonadal and antigonadal activities, depending upon the particular circumstances under which it is administered. The activity of the pineal gland is under the influence of the light:dark cycle. The predominant role of light restriction seems to be stimulatory in both humans and other animals, whereas light exposure is inhibitory. Hence, the pineal gland is a neuroendocrine transducer, converting cyclic nervous activity entrained by cyclic environmental illumination into endocrine information (Quay, 1963; Wurtman et al., 1963; Axelrod et al., 1965; Axelrod, 1974).

Innervation of the Pineal Gland:

Three groups of nerve fibers have been described in the mammalian pineal gland: sympathetic, parasympathetic and commissural fibers. Among these only the sympathetic nerve fibers have been clearly shown to be of importance for the proper functioning of the pineal gland in terms of its response to the photoperiod (Kappers, 1960a,b; 1964; 1965; Wurtman et al., 1968).

The neural connection between the eyes and the pineal in rodents is illustrated in Fig. I-2. Axons of retinal ganglion cells enter the optic nerve and either subserve visual processes or neuroendocrine events. Those retinal fibers that subserve neuroendocrine events synapse in the suprachiasmatic nuclei (Moore and Lenn, 1972; Moore, 1973; Moore and Klein, 1974). A multisynaptic pathway connects the suprachiasmatic nuclei to the preganglionic sympathetic cell bodies in the intermediolateral cell columns of the upper thoracic cord. Neurons originating in the intermediolateral columns provide the preganglionic connections to the superior cervical ganglia from which originate the postganglionic sympathetic nerves which enter the tentorium cerebelli and eventually form one or two relatively discrete nerves, the nervi conarii which enter the pineal gland. The nerve fibers in the pineal gland terminate in the vicinity of the

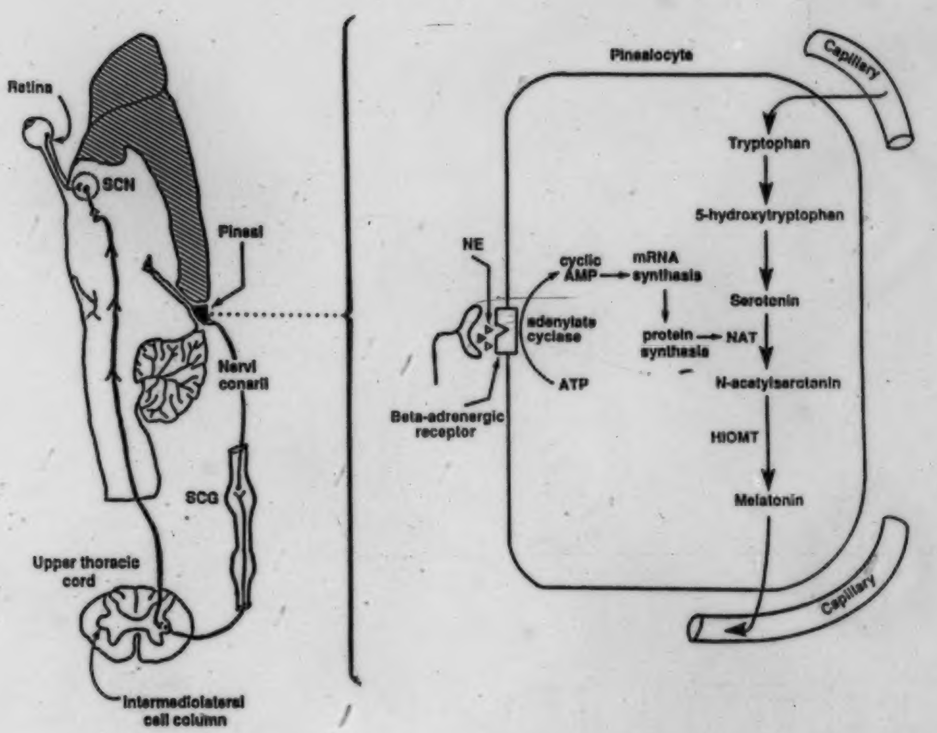
Retina
SCN
Pineal
Nervi conarii
SCG
Upper thoracic cord
Intermediolateral cell column
Pinealocyte
Capillary
Tryptophan
5-hydroxytryptophan
NE
cyclic AMP
mRNA synthesis
Serotonin
adenylate cyclase
protein synthesis
NAT
ATP
N-acetylserotonin
Beta-adrenergic receptor
HIOMT
Melatonin
Capillary

Fig. I-2. Schematic diagram of the neuroanatomical connections from the retina to the pineal gland, and the sequence of biochemical events in the synthesis of melatonin after adrenergic stimulation of the pinealocytes. HIOMT, hydroxyindole-O-methyltransferase; NAT, N-acetyltransferase; NE, norepinephrine; SCG, superior cervical ganglion; SCN, suprachiasmatic nucleus.

pinealocytes. These fibers and their transmitter substance norepinephrine (NE) are important for the regulation of pinealocyte function (Kappers, 1965; Wurtman and Axelrod, 1965; Moore, 1978).

Biochemistry and Control of Secretion:

The mammalian pineal gland is known to contain a number of substances which have been either demonstrated or postulated to be synthesized within the pineal gland and which may exert their primary action at a site distant from the pineal gland. The best known of these pineal substanc~~es is~~ the indole melatonin, though other related substances are present here. The mammalian pineal gland also contains a great number of peptides such as arginine vasotocin, oxytocin and several other peptides, polypeptides and protein fractions (Quay, 1981; Reiter et al., 1981b; Ebels, 1982).

Photic and Neural regulation:

Melatonin, the best known substance of the pineal gland, was first isolated from bovine pineal gland in 1959 by Lerner and his associates and was chemically identified as N-acetyl-5-methoxytryptamine.

The synthesis of melatonin within the pineal gland is regulated by external photoperiodic cues and by internal hormonal feedback (Reiter, 1986b; Cardinali, 1981a). In mammals, the daily cycle of light and darkness appears to synchronize the biosynthetic activity and endocrine function of the pineal gland. In all mammalian species tested, whether nocturnally or diurnally active, serum melatonin exhibits a distict circadian rhythm; night time levels are several times higher than the daytime levels (Brown and Niles, 1982; Reiter, 1986a,b).

As described above, the neural message reaches the pineal gland via the postganglionic sympathetic fibers which terminate in the pineal gland. Exposure to darkness stimulates the postganglionic sympathetic fibers to release norepinephrine which interacts with β-receptors and possibly also with α-receptors on the pinealocyte membrane (Cardinali, 1981b, 1983; Vacas et al., 1985) to stimulate adenylate cyclase and the formation of cyclic-AMP. The increase in cyclic-AMP is followed by a rise in messenger RNA and protein synthesis with a resultant increased activity of the enzyme N-acetyltransferase (NAT) (Klein et al., 1970; Buda and Klein, 1978). The NAT exhibits a rhythm of activity (dark-time peak) which is believed to be responsible for circadian rhythms in melatonin production. This view stems from findings that the increase in NAT during the nighttime is directly correlated with a nighttime elevation in pineal and serum melatonin (Rudeen and Reiter, 1977, 1979; Binkley, 1983; Ho et al., 1984; Reiter et al., 1984; Reiter, 1986b).

Pineal biosynthesis of melatonin is initiated by the uptake of circulating L-tryptophan (Fig. I-2) which is the common precursor of pineal and brain indoles. L-tryptophan is hydroxylated in pinealocytes by tryptophan hydroxylase to produce 5-hydroxytryptophan (Lovenberg et al., 1967). The resulting compound is then acted upon by 5-hydroxytryptophan decarboxylase to produce 5-hydroxytryptamine or serotonin (Lovenberg et al., 1962; Snyder and Axelrod, 1964, 1965). Under the influence of NAT, serotonin is converted to N-acetylserotonin (Weissbach et al., 1960, 1961). This compound is then O-methylated through the action of hydroxyindole-O-methyltransferase (HIOMT) to N-acetyl-5-methoxytryptamine (melatonin) (Axelrod and Weissbach, 1960, 1961).

Besides melatonin, there are a number of other indoles produced in the pineal gland. For example, serotonin can be metabolized to other indoles such as 5-hydroxytryptophol. This can be methylated in the presence of HIOMT to 5-methoxytryptophol (Wurtman et al., 1968; Klein et al., 1981; Reiter et al., 1981b).

Melatonin is released shortly after its synthesis, primarily into the blood vascular system (Kennaway et al., 1977; Cardinali, 1981b) and in some cases directly into the cerebrospinal fluid (CSF) (Hedlund et al., 1977; Reppert et al., 1979). Melatonin concentrations in the blood and CSF follow a daily rhythm, with highest concentrations occuring during the night and lowest levels during the day.

Pineal and Reproduction:

The primary effect of the pineal relative to the reproductive system is suppressive. Knowledge of the antireproductive effects of the pineal gland stemmed from earlier clinical observations in which the precocious syndrome was in some cases associated with pineal tumors. It has been observed that this syndrome is usually present with non-parenchymatous tumors that destroy the pineal gland, whereas parenchymatous tumors display a high degree of association with depressed gonadal function (Relkin, 1976).

In 1959, Wurtman and associates suggested that the pineal may secrete a substance which inhibits some aspects of gonadal function. Now it is well established that the pineal secretory products influence the structure and function of the reproductive organs via an effect on the hypothalamic-pituitary-gonadal axis. As mentioned earlier in this report, the primary regulatory influence of the pineal gland is photoperiodic information. Under the influence of short days, the pineal gland is maximally stimulated with the resultant production and secretion of antireproductive hormones. In the absence of the pineal gland, darkness is incapable of inducing gonadal regression (Reiter and Hester, 1966).

Reproductive Effects in Rodents:

Different species of mammals vary considerably in their response to the antagonistic action of the pineal gland when the latter is stimulated by light deprivation. The Syrian golden hamster (*Mesocricetus auratus*) is very sensitive to the absence of photic stimuli. Therefore, this species is well suited for studies dealing with the interactions of the photoperiod and reproduction.

Adult male and female Syrian hamsters require in the order of 12.5 hours of light per day if the reproductive organs are to remain in a mature state (Gaston and Menaker, 1967; Elliott, 1976). Exposure of adult male hamsters to a photoperiod of less than 12.5 hours light/24-h period (Gaston and Menaker, 1967; Elliott, 1976; Stetson and Tate-Ostroff, 1981) or depriving them of photic information such as by blinding or exposing them to darkness (Hoffman and Reiter, 1965; Reiter and Hester, 1966; Reiter, 1967; Gravis, 1978; Demaine et al., 1983) results in reproductive collapse in approximately 9 weeks. This collapse is evident by a substantial decline in the weights of the testes and accessory sex glands (seminal vesicles and coagulating glands).

The testes and accessory sex organs of male hamsters maintained in short days, exhibit morphological changes normally associated with sexual incompetence. Characteristically, the atrophied testes of such animals exhibit pronounced involution of the Leydig cells, and the seminiferous tubules are devoid of spermatozoa. A study by Lin and Wing (1978), who examined the effects of blinding on the fine structure of the Leydig cells and seminal vesicles, indicated that all cytological indicators of secretory activity associated with protein synthesis are depressed in the secretory epithelial cells of the seminal vesicles. In these animals, the Leydig cells are also extremely regressed as compared to intact animals maintained in long daily photoperiod. Our previous study (Buzzell et al., 1985), using light microscopic stereological techniques, indicated that exposure of adult male hamsters to short-day photoperiods results in minimal changes in the structural integrity of the ventral lobes of the prostate, compared with intact animals kept in long days. In the dorsal prostate, on the other hand, there are increases in the proportion of nonepithelial elements such as a large increase in the relative amount of interacinar tissue and, within the acini, large increases in the relative contributions of the lamina propria and muscular stroma and decreases in the volume fraction of lumen, compared with intact animals maintained in long days.

In addition to the decline in the weights of the testes and accessory sex glands, light-restriction decreases both pituitary content (Reiter and Johnson, 1974a) and serum (Reiter, 1973; Berndtson and Desjardins, 1974; Turek et al., 1975; 1976; Tamarkin et al., 1976; Goldman and Brown, 1979; Steger et al., 1984) levels of LH and FSH; pituitary and serum levels of prolactin (Reiter and Johnson, 1974a; Reiter, 1975, 1980a; Matt and Stetson, 1980; Borer et al., 1982); and circulating levels of testosterone (Berndtson and Desjardins, 1974; Pickard and Silverman, 1979).

Light restriction by any of several means has a similar depressive influence on the reproductive system of female hamsters. The uteri become infantile, the ovaries become incapable of producing mature gametes and become devoid of corpora lutea (Hoffman and Reiter, 1966; Reiter, 1969; Jorgenson and Schwartz, 1985). Furthermore, normal vaginal cyclicity ceases (Sorrentino and Reiter, 1970; Bridges and Goldman, 1975; Seegal and Goldman, 1975; Jorgenson and Schwartz, 1985). However, unlike the male hamster, the pituitary and circulating levels of LH and FSH increase (Reiter and Johnson, 1974b; Bridges and Goldman, 1975; Seegal and Goldman, 1975; Goldman and Brown, 1979). Female hamsters with photoperiod-mediated gonadal atrophy exhibit a daily afternoon surge of LH and FSH which is not followed by ovulation (Bridges and Goldman, 1975; Seegal and Goldman, 1975). This is in contrast to the situation in females exposed to long daily photoperiods, where surges in LH and FSH occur only every fourth day and are usually followed by ovulation. Light deprivation in female hamsters has also been found to significantly depress pituitary prolactin (Reiter and Johnson, 1974b).

The effects of light-restriction upon the hypothalamic-pituitary-gonadal axis of male and female hamsters can be prevented if the pineal gland is removed prior to blinding or exposure to short days (Reiter and Hester, 1966; Demaine et al, 1983). Furthermore, interference with the sympathetic innervation of the pineal gland, i.e., surgical interruption of the nervi conarii or extirpation of the superior cervical ganglia, completely eliminates gonadal regression in hamsters subjected to blinding or exposure to short days (Reiter, 1972). This indicates that the effects of light restriction on the reproductive status of these animals are mediated by the pineal gland.

Pineal-mediated reproductive collapse can also be induced in the rat. However, the reproductive consequences of simple light restriction in the rat are minimal. Therefore, several experimental manipulations have been found which, although by themselves incapable of inducing severe gonadal regression, do result in severe antigonadal consequences when combined with light deprivation. These experimental manipulations are referred to as potentiating factors and include early neonatal injection of gonadal steroids (Reiter et al., 1968a,b,c); surgical removal of the olfactory bulbs, thus rendering the animals anosmic (Reiter et al., 1969; Reiter and Ellison, 1970) or restricting the food intake (Sorrentino et al., 1971). It has been suggested that these factors increase the sensitivity of the inhibited site to the pineal antireproductive factors (Reiter, 1981; Reiter et al., 1969, 1971; Klein et al., 1971).

When rats are dual sensory deprived, i.e. rendered both blind and anosmic, the reproductive system undergoes severe collapse. In male rats, the testes, seminal vesicles, coagulating glands and ventral lobes of the prostate are found to atrophy (Reiter, et al., 1971, Mediavilla et al., 1985; Sanchez-Barcelo et al., 1985). Buzzell et al. (1984) found in blind-anosmic male rats decreases in the weights of the ventral prostate and the volumes of the acini, reflecting significant decreases in the diameter of acini. The atrophic responses of these organs do not develop in similarly treated pinealectomized rats, indicating that the results of blinding and anosmia are pineal-mediated phenomena (Reiter, et al., 1971; Buzzell et al., 1984; Mediavilla et al., 1985).

Hormonal imbalance has been observed in dual sensory deprived male and female rats. A number of studies have indicated that in male rats blinding and anosmia results in a decrease in pituitary content of LH and prolactin (Reiter, et al., 1975; Chen and Reiter, 1980). Blind and anosmic rats also showed a marked reduction in circulating levels of LH (Reiter et al., 1975; Ronnekleiv and McCann, 1975; Chen and Reiter, 1980), prolactin (Chen and Reiter, 1980, Leadem and Blask, 1982a) and testosterone (Mediavilla et al., 1985; Sanchez-Barcelo et al, 1985).

The reproductive hormones governing the functions of the ovaries and uteri in female rats change after experimental manipulation of the physiology of the pineal gland. Blind and anosmic female rats exhibit a substantial decrease in pituitary and serum levels of LH and FSH along with a decline in the synthesis, storage and release of pituitary prolactin (Blask and Reiter, 1975; Leadem and Blask, 1982a,b). In a related study, Leadem and Blask (1982c) reported that blinding combined with anosmia actually decreased the size of the pars distalis of the pituitary gland in female rats and caused a reduction in the number and size of prolactin secreting cells, effects which could be prevented by pinealectomy.

Thus, the mammalian pineal gland, when appropriately challenged, may have a profound influence on the neuroendocrine-reproductive axis and leads to involution of reproductive organs, including accessory sex glands. Both indoles and polypeptides isolated from the pineal gland have been implicated as possible mediators of pineal antireproductive function. Several methoxyindoles and a number of other compounds such as peptides appear to possess antireproductive properties. The indoles have been tested under a greater variety of physiological conditions than have the peptides. The indole most frequently examined for its reproductive effects has been melatonin. A number of studies have suggested that melatonin produces the antireproductive effects associated with light restriction-induced pineal stimulation.

A number of studies have been designed to establish the target site for melatonin. Many regions, both within and outside the nervous system, may be sites of action. These include the hypothalamus, midbrain, pituitary gland, gonads, accessory sex organs, peripheral nerves and the pineal gland itself (Fig. I-3).

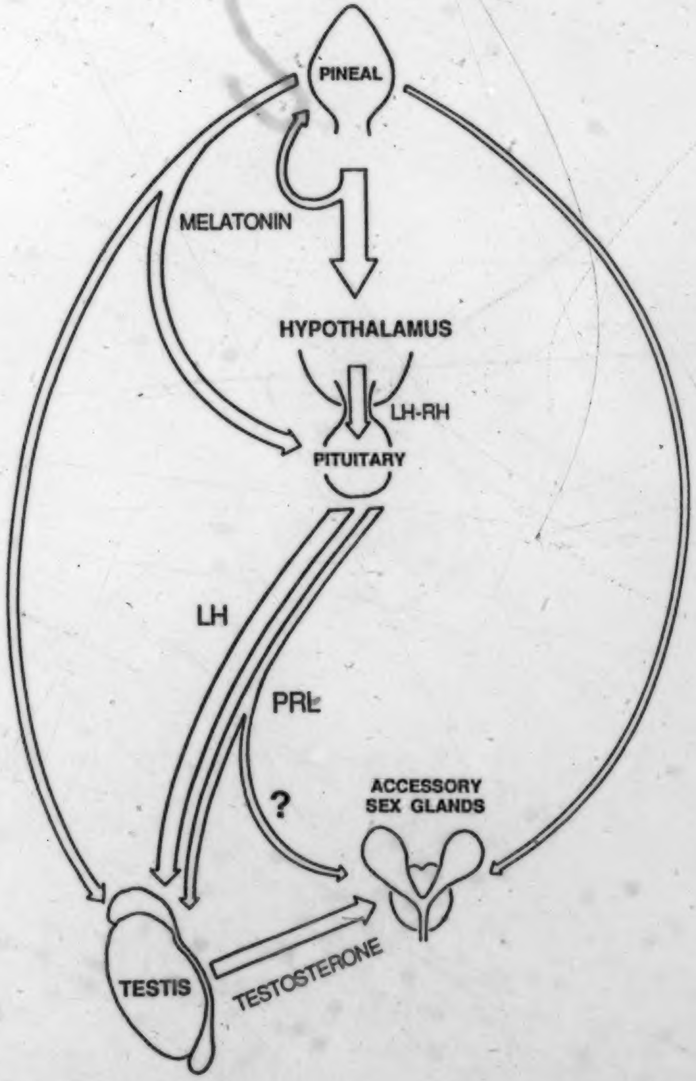
PINEAL
MELATONIN
HYPOTHALAMUS
LH-RH
PITUITARY
LH
PRL
?
ACCESSORY SEX GLANDS
TESTIS
TESTOSTERONE

Fig. I-3. Diagrammatic representation of the hormonal control of male reproductive organs, and of the potential sites of action of pineal melatonin.

Several studies have implicated the hypothalamus as the primary site of action of pineal indoles. Hypothalamic melatonin implants, suprachiasmatic lesions and surgical deafferentation studies support this concept (Reiter et al., 1983). Crystalline melatonin implanted stereotaxically into the median eminence or into the reticular formation of the mesencephalon leads to a dramatic reduction in the LH content of the anterior pituitary (Fraschini et al., 1971). Likewise, when pineal fragments were implanted into the median eminence (Fraschini et al., 1971), pituitary LH content was decreased. Therefore, it has been concluded that melatonin suppresses LH production and secretion by its action on specific receptors in the median eminence.

Melatonin may also alter the number or sensitivity of hypothalamic steroid receptors, or intracellular processing of steroid-receptor complex (Blask, 1981). It may increase the sensitivity of the hypothalamic-pituitary axis to the negative feedback effects of androgens (Tamarkin et al., 1976; Turek, 1977).

In a study by Cardinali and Freire (1975), melatonin treatment of adult male rats was found to interact with microtubular protein in the hypothalamic tissue. These investigators reported that melatonin treatment decreased microtubule protein content by 44% in the arcuate-median eminence region and by 19% in the remaining hypothalamic block. Superior cervical ganglionectomy, on the other hand, increased microtubule protein content of the rat hypothalamus. As microtubules have been implicated in several types of intracellular movement including the transport and release of various secretory products, such as hormones and neurotransmitters, it has been suggested that the decrease in the microtubular protein induced by melatonin may be a mechanism by which this indole modulates the release of gonadotropin-releasing hormones (Blask, 1981).

Anterior hypothalamic deafferentation has also been shown to prevent the antigonadotrophic effects of melatonin (Reiter et al., 1981a) which suggests that injections of melatonin inhibit reproductive function by an action on the central nervous system. Reiter et al. (1981a) suggested that melatonin may act on neurons located outside the medial basal hypothalamus (MBH) whose axons enter the MBH anteriorly. One possible site that was proposed was the surachiasmatic nuclei. In their experiment, the connections of the suprachiasmatic nuclei to the MBH were severed.

Testing the possibility that pineal indolic compounds directly affect basal pituitary gonadotropin synthesis or release, several studies have indicated that intrapituitary implants (Fraschini et al., 1968) or infusion (Kamberi et al., 1970; 1971) of melatonin and related indoles in intact adult rats had no effect on pituitary LH reserves, and LH, FSH and prolactin release. The study by Wun et al. (1986a,b), on the other hand, indicated that melatonin had direct inhibitory effects upon the release of FSH and LH from hamster pituitary glands.

The antigonadotrophic effects of pineal indolic compounds may also be exerted, in part, at the peripheral reproductive level. Since the gonads and accessory sex organs undergo marked regression associated with increased pineal activity, it has been proposed that these organs may represent a principle site of action of the pineal hormone.

Debeljuk et al. (1971) examined the effect of melatonin in hypophysectomized, human chorionic gonadotropin (hCG) treated rats. Melatonin hastened the regression of the testes and accessory sex organ weights that is normally seen after hypophysectomy. Melatonin was also effective in lowering the weights of the testes and accessory sex organs in hypophysectomized rats that were treated with hCG. In a related study, Debeljuk et al. (1970) found that melatonin was effective in restricting the growth of the accessory sex organs in castrated rats in response to testosterone administration. Trentini et al. (1976) indicated that ovarian uptake of ^{3}H-LH was reduced in rats pretreated with melatonin, suggesting that binding of LH to its receptors was inhibited. Shirama et al. (1981) reported that the changes in the weights of the accessory sex glands (ventral lobes of the prostate and seminal vesicles) of rats exposed to continuous darkness were not correlated with the plasma gonadotropins and testosterone levels, compared with pinealectomized rats exposed to the same photoperiod, suggesting a direct effect of the pineal on the accessory sex glands. Finally, Cohen et al. (1978a,b) found alleged cytoplasmic melatonin receptors in gonads of several species, including human. These results support the hypothesis that melatonin may exerts direct regulatory actions on peripheral reproductive target organs.

R

Another site of action of melatonin could be the pineal gland itself. It has been postulated that melatonin is a releasing factor for other pineal antigonadotrophic substances. For example intraventricular or intracarotid injections of melatonin in cats induced the release of an arginine vasotocin-like substance into the CSF and plasma (Pavel, 1973; Pavel and Goldstein, 1979).

It can be concluded that most components of the neuroendocrine-reproductive axis may represent target sites for pineal hormones.

It is clear from the foregoing review that the mammalian pineal gland has endocrine capabilities. It may modulate the endocrine system and has an especially profound controlling influences on the neuroendocrine-reproductive axis. Involution of the testes and accessory sex glands is characteristic of common laboratory rodents which are completely deprived of light or placed in a short day photoperiodic environment. This involution is blocked by removal or denervation of the pineal gland. Consistent with these changes are alterations in the circulating reproductive hormones, such as the gonadotropins, prolactin, and androgens.

Present Study

Although several reports dealing with effects of light-restriction on reproductive organs have appeared in the literature, in most of these studies the weight of the gonads and accessory sex glands were used as indications of the effects on reproductive organs. In male rodents, the accessory sex glands weighed have usually been the seminal vesicles and coagulating glands. The effects of photoperiods on the weights of other accessory sex glands and on the fine structure and biochemical components of these organs have not been studied, except by Lin and Wing (1978), who examined the effects of photoperiod and pinealectomy on the fine structure of the seminal vesicles of Syrian golden hamster, and that reported by Lucini et al. (1983) who examined the effects of photoperiods on testosterone metabolism *in vitro* by the prostate gland. Furthermore, while it is generally assumed that the effects of photoperiod on the accessory sex glands in male rodents are due to a decrease in circulating testosterone levels, this has not been rigorously examined.

The research outlined in this thesis was designed to rectify these deficiencies. There were three goals: 1) to determine the effects of photoperiod on the weights of the major accessory sex glands, such as the ventral and dorsal lobes of the prostate, coagulating glands, ampullary glands and seminal vesicles; 2) to determine the effects of photoperiod on the fine structure of accessory sex glands; and 3) to determine the effects of photoperiod, combined with other endocrine manipulations on accessory sex glands in order to try to sort out direct from indirect effects.

In order to meet these overall goals, the following studies were carried out to examine:

1- The effects of short-day exposure and pinealectomy on the weights of the major accessory sex glands.

2- The effects of short day-mediated by the pineal gland on the fine structure of the ventral and dorsal lobes of the prostate.

3- The effects of short days and castration in short and long days on the accessory sex glands.

4- The effects of short days on the accessory sex glands in the presence of excess prolactin.

5- The effects of photoperiod and the pineal gland on the weight, protein and DNA content of the accessory sex glands in the presence of levels of testosterone capable of maintaining the size of reproductive organs in castrated hamsters.

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II- EFFECTS OF PHOTOPERIODS ON ACCESSORY SEX GLANDS OF THE SYRIAN HAMSTER

INTRODUCTION

Photoperiod is an environmental cue used by many mammals to time their seasonal reproduction. For instance, the Syrian hamster, *Mesocricetus auratus*, is a highly photosensitive species, and its reproductive system is very sensitive to environmental lighting conditions. Therefore, this species has been useful in defining the role of the photoperiod in the control of reproductive physiology.

It is well known that the reproductive organs of the adult male Syrian hamster undergo regression when the animal is exposed to a short photoperiod, i.e. less than 12.5 h light/day (Gaston and Menaker, 1967; Elliott, 1976; Stetson and Tate-Ostroff, 1981), or when it is deprived of photic information by blinding or exposing it to darkness (Hoffman and Reiter, 1965; Reiter and Hester, 1966; Reiter, 1967; Gravis , 1978). The atrophied testes of such animals are devoid of any mature spermatids and have small Leydig cells (Reiter and Hester, 1966; Reiter, 1967; Gravis, 1978; Lin and Wing, 1978; Demaine et al., 1983). Paralleling the involution of the testes, the accessory sex glands are smaller in weight than those of animals kept in long days. However, in most of these studies, the accessory sex glands weighed were the seminal vesicles and coagulating glands. The purpose of the present study was to determine what effects short-day photoperiods have on the weights of gonads and all of the major accessory sex glands, and on levels of reproductive hormones in adult male Syrian hamsters.

MATERIALS AND METHODS

Male Syrian hamsters, *Mesocricetus auratus*, were obtained at ages of 7-8 weeks from the Charles River Breeding Laboratories, Montreal. After arrival in Edmonton, they were housed in groups of 3-4 animals in clear plastic cages under controlled condition of light (14 h light/day, lights on 0600-2000 h) and temperature (22-25 °C) in the University of Alberta Medical Sciences Building Animal Unit. They were provided with a standard pellet diet and tap water *ad libitum*. Acclimation for at least one week under the environmental conditions mentioned above, was allowed prior to experimentation.

Twenty adult male hamsters were used in this study. Half were maintained in short-day photoperiods (10h:14h L:D, lights on 0800-1800 h) to examine the antireproductive effects of short-day exposure, and the other half were kept in long-day photoperiods (14h:10h L:D, lights on 0600-2000 h) to act as controls for the effects of short days.

After 10 weeks of exposure to these lighting regimes, each hamster was weighed and decapitated. Trunk blood was collected in heparinized tubes and plasma was obtained by centrifugation (IEC HN-SII Centrifuge) at 2500 r.p.m. for 10 minutes. The plasma was stored in several aliquots of 200-500 μl volume at -70 °C for subsequent hormone assays.

The testes and the accessory sex glands were dissected free of surrounding tissue and weighed.

Plasma levels of testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactin were determined by double antibody radioimmunoassay procedures. The assay was done in the laboratory of Dr. R.J. Reiter, University of Texas, Health Science Center at San Antonio, Texas by Dr. G.R. Buzzell (testosterone, LH and FSH) and M.K. Vaughan (Prolactin). Testosterone levels were determined using the Coat-A-Count Kit supplied by the Diagnostic Product Corporation, Los Angeles, CA., with human-based standards. Reagents for the assay of LH and FSH were provided by the Rat Hormone Distribution Program, NIAMDD, NIH, Maryland. Plasma prolactin levels were measured using a homologous radioimmunoassay and are expressed in terms of a purified hamster prolactin preparation.

The data were statistically analyzed by Student's t test. A value of $P < 0.05$ was considered to indicate significant differences between short and long days.

RESULTS

The effects of exposure to long-day and short-day photoperiods on the weights of the reproductive organs, and on circulating levels of reproductive hormones are presented in Fig. II-1. Subjecting adult male hamsters to short-day photoperiods for 10 weeks, resulted in a significant reduction in the weights of the testes and of all accessory sex glands compared to those of animals maintained in

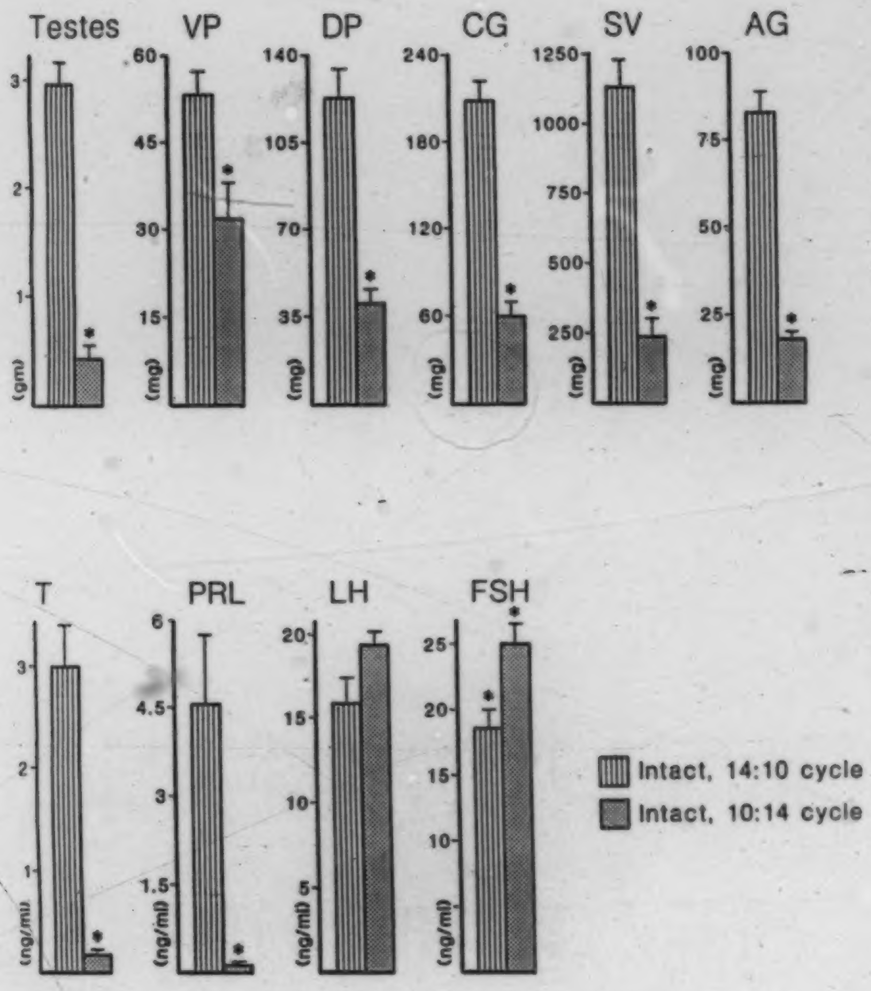

Fig. II-1. Testicular and accessory sex organ weights and plasma concentrations of reproductive hormones in intact adult hamsters kept under either long-day (14:10 cycle) or short-day (10:14 cycle) photoperiods. VP, ventral lobes of the prostate; DP, dorsal lobes of the prostate; CG, coagulating glands; SV, seminal vesicles; AG, ampullary glands; T, testosterone; PRL, prolactin; LH, luteinizing hormone; FSH, follicle-stimulating hormone. Data are mean ± standard error of the mean (vertical lines). $^*P < 0.05$.

long-day photoperiods. Amongst the accessory sex glands, the ventral lobes of the prostate showed the least response to short days; the mean weight of the ventral lobes of the prostate in hamsters maintained in short days was about 59% of that maintained in long days, whereas the weights of the dorsal lobes of the prostate, coagulating glands, seminal vesicles and ampullary glands of hamsters in short days were about 33%, 28%, 21% and 22% of those maintained in long days respectively.

Short-day exposure, while leading to a significant reduction in circulating testosterone and prolactin levels, had no effect upon LH levels, whereas FSH levels were significantly higher than those of animals maintained in long days.

DISCUSSION

Exposure of adult male Syrian hamsters to short-day photoperiods for 10 weeks resulted in a marked reduction in the weight of the testes and accessory sex glands, compared with those of animals kept in long photoperiods. This supports and extends earlier studies indicating that reduced weights of the gonads, coagulating glands and seminal vesicles are the usual consequences of short-day exposure (Hoffman and Reiter, 1965; Gaston and Menaker, 1967; Reiter, 1975; Elliott, 1976; Pickard and Silverman, 1979; Steger et al., 1982, 1983). Nevertheless, in response to short day exposure, the ventral lobes of the prostate lost less weight than did other accessory sex glands. These results confirm our earlier observations reported by Buzzell et al. (1985) who examined the effects of long- and short-day photoperiods on the structural components of the ventral and dorsal lobes of the prostate in intact hamsters. In their study, the ventral lobes of the prostate reacted to short days with minor morphological changes relative to those observed in the dorsal lobes.

The atrophy of the reproductive organs in the present study, was accompanied by a significant drop in circulating levels of testosterone and prolactin, but neither circulating levels of LH nor FSH were reduced. Although several reports have indicated that atrophy of reproductive organs caused by short photoperiods is usually accompanied by decreases in circulating levels of LH and FSH (Reiter, 1973; Berndtson and Desjardins, 1974; Turek et al., 1975, 1976; Tamarkin et al., 1976; Goldman and Brown, 1979; Steger et al., 1984), prolactin (Matt and Stetson, 1980; Reiter, 1980; Borer et al., 1982), and testosterone (Berndtson and Desjardins, 1974; Pickard and Silverman, 1979), the results obtained from the present study and those reported by other investigators (Reiter, 1975; Bex et al., 1978; Steger et al., 1985) suggest that a decline in peripheral levels of LH and FSH does not occur consistently in hamsters kept in short days. Despite the fact that gonadotropins are important for the maintenace of the reproductive organs weight and function, it has been reported that daily injection of synthetic luteinizing hormone-releasing hormone (LH-RH) did not prevent the atrophy of reproductive organs in light-deprived animals, even though LH-RH stimulated the release of both LH and FSH (Chen and Reiter, 1980).

In male hamsters, prolactin also appears to be essential for the maintenance of normal reproductive size (Bartke et al., 1975, 1979;

Chen and Reiter, 1980). Prolactin has been shown to influence the number of available LH receptors in the testes (Bex and Bartke, 1977). Thus the significant decrease in circulating prolactin levels seen in the present study in animals maintained in short days, may affect the action of LH at the testicular level resulting in the observed significant reduction in testosterone levels and the subsequent reduction in the weights of the accessory sex glands, which are extremely sensitive to androgen deprivation (Price and Williams-Ashman, 1961; Williams-Ashman, 1964).

A number of studies strongly suggest that the effects of short photoperiods on the reproductive system of hamster are primarily mediated by the pineal gland, since these changes do not occur in the absence of an intact and sympathetically innervated pineal gland (Hoffman and Reiter, 1965; Reiter, 1967, 1980, 1983; Steger et al., 1984). Since the biosynthesis of melatonin in the pineal gland is controlled by the lighting schedule, this indole may be the pineal hormone that modulates the status of the reproductive system via its action on the neuroendocrine-pituitary-gonadal axis, or by acting directly on the accessory sex glands (Blask, 1981; Shirama et al., 1981; Reiter, 1982).

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III- IS REMOVAL OF THE PINEAL GLAND EFFECTIVE IN PREVENTING SHORT DAYS-INDUCED ACCESSORY SEX GLANDS REGRESSION?

INTRODUCTION

The results obtained from the previous experiment showed that short-day exposure resulted in a significant reduction in the weights of the testes and of all the major accessory sex glands, including the ventral and dorsal lobes of the prostate. In several mammalian photoperiodic species, the pineal gland has been shown to play an important role in mediating the effects of photoperiod on reproductive physiology (Reiter, 1980, 1987). In Syrian hamsters, removal of the pineal gland prevents the antireproductive effects of short days (Reiter and Hester, 1966; Demaine et al., 1983). Therefore, the present study was carried out to confirm that removal of the pineal gland would prevent the regression of the testes and accessory sex glands. I was interested particularly in the ventral and dorsal lobes of the prostate which have not been previously examined in pinealectomized hamsters maintained in short days.

MATERIALS AND METHODS

In this experiment, 47 adult male Syrian hamsters were used (for supply and care of animals, see Chapter II). They were divided into two major groups. Hamsters in the first group (24 animals) were pinealectomized as outlined below. Those of the second group (23 animals) were subjected to a sham pinealectomy, to establish that the results obtained were due to the effects of pinealectomy and not to the surgical trauma.

Pinealectomy was performed according to the method of Hoffman and Reiter (1965b). Each animal was anesthetized by inhalation of Metofane (M.T.C. Pharmaceuticals, Mississauga, Ontario), and mounted with head kept immobilized on a stereotaxic surgical base. A median incision was made in the skin between the ears, and the skin was reflected. Subcutaneous connective tissue was then scraped from the skull. By using a small disc drill, a disc of bone 5 mm in diameter was removed from over the confluence of the sagittal and transverse sinuses, and the pineal was removed with fine forceps passed through the sinuses. The bone disc was replaced and the wound was closed by surgical clips. Sham pinealectomy included the skin incision, drilling the bone over the confluence of the sinuses, and closing the wound by surgical clips. After surgery, all animals were allowed to recover for at least one week before the onset of the experiment.

One week after the surgery, 12 animals of the pinealectomized and 11 of the sham pinealectomized groups were placed in short-day photoperiods (10h:14h, L:D) (experimentals) while the remainder of each group was kept in long-day photoperiods (14h:10h, L:D) (controls). The room temperature was maintained at 22-25 °C. After 10 weeks of exposure to the above lighting conditions, each hamster was weighed, decapitated, and trunk blood was collected into heparinized tubes for plasma separation. The testes and all the accessory sex glands were removed and weighed. Plasma levels of testosterone, LH, FSH and prolactin were measured in pinealectomized animals maintained in short and long-day photoperiods as outlined above in Chapter II.

Statistical comparisons of the data were carried out by one-way analysis of variance and Student's t-test. Significance levels were set at $P < 0.05$.

RESULTS

Exposure of sham pinealectomized animals to short-days led to a significant reduction in the weights of the testes, dorsal lobes of the prostate, coagulating glands, seminal vesicles and ampullary glands, compared to pinealectomized animals maintained in long or short days, or sham pinealectomized ones kept in long days (Fig. III-1). None of the treatment procedures employed had any statistically significant influence on the weights of the ventral lobes of the prostate.

The plasma testosterone, LH, FSH and prolactin levels in pinealectomized hamsters kept in short days, did not differ significantly from those of the pinealectomized ones kept in long days (Table III-1).

In hamsters maintained in long days, pinealectomy was without effect on all reproductive parameters, compared with sham pinealectomized animals kept in the same photoperiods.

Among the 12 hamsters that were subjected to sham pinealectomy and kept in short days, 2 hamsters showed large testes and accessory sex glands relative to the other hamsters subjected to a similar treatment. However, the results obtained from these animals were also included in this study.

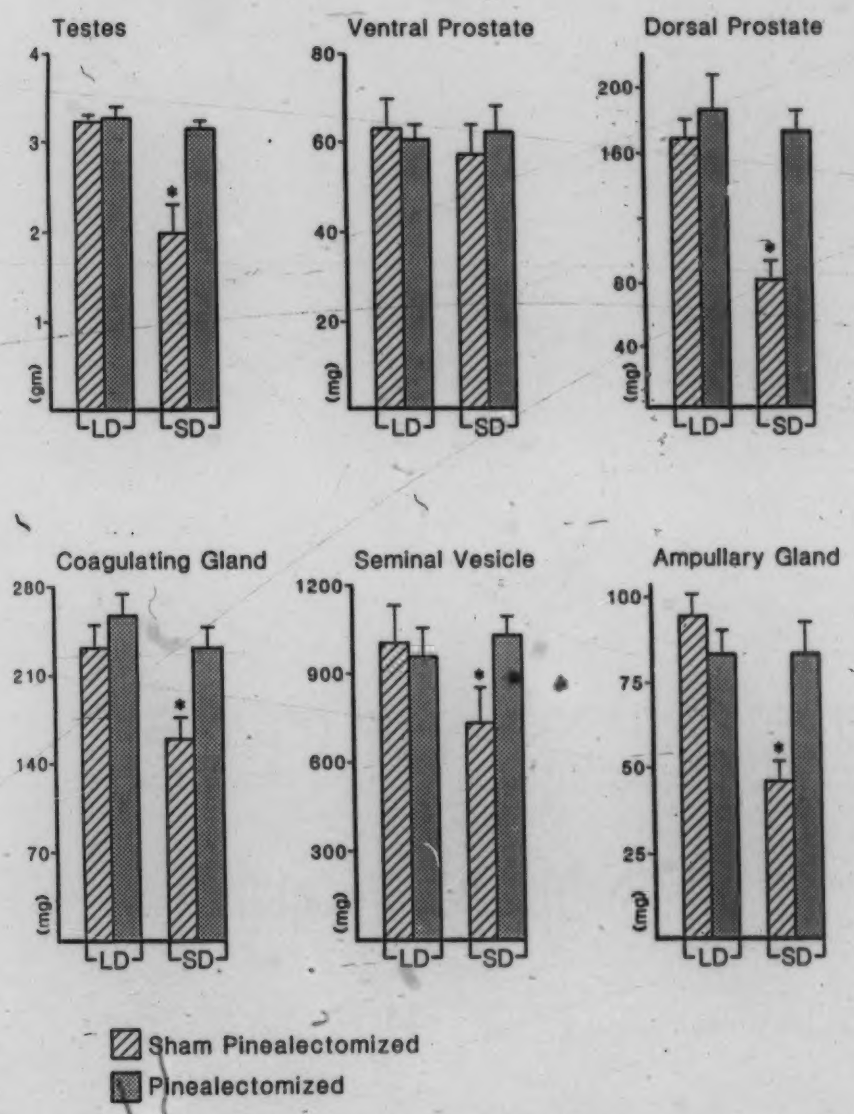

Fig. III-1. Testicular and accessory sex organ weights in sham-pinealectomized and pinealectomized hamsters kept under either long-day (LD) or short-day (SD) photoperiods. Data are mean ± standard error of the mean (vertical lines). $^{*}P < 0.05$, *vs*. all other groups.

Table III-1. Plasma concentrations of testosterone (T), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactin (PRL) in pinealectomized (PINX) hamsters kept under long-day (14:10) or short-day (10:14) photoperiods.

Treatment	T ng/ml	LH ng/ml	FSH ng/ml	PRL ng/ml
PINX, 14:10	2.77 ± 0.32 (12)	14.05 ± 1.38 (12)	18.13 ± 3.34 (12)	4.69 ± 0.96 (11)
PINX, 10:14	1.82 ± 0.46 (11)	13.12 ± 1.83 (12)	11.82 ± 1.12 (11)	6.08 ± 2.48 (10)

Data are the mean ± standard error of the mean.
The number of samples is in parenthesis.

DISCUSSION

In sham-operated hamsters that were maintained in short daily photoperiods, the testes and the accessory sex glands, except the ventral lobes of the prostate, showed characteristic atrophic responses to short days exposure. It appears that the response of the accessory sex glands of hamsters maintained in short days, which usually show a significant decline in circulating testosterone levels [Berndtson and Desjardins, 1974; Pickard and Silverman, 1979; (Chapter II)], is similar to that observed in androgen deprived hamsters induced by castration. Ortiz (1953) and Schmidt and Visek (1977) indicated that among the accessory sex glands that were examined, the ventral lobes of the prostate showed the least response to castration. Ortiz (1953) noted that this gland regressed very slowly after pubertal or adult castration, and the degree of regression was proportional to the length of castration period.

In the present study, removal of the pineal in hamsters kept in short daily photoperiods negated the suppressive effects of short days on the weights of the gonads and accessory sex glands including the weight of the dorsal lobes of the prostate and ampullary glands which have not been previously described. Likewise, the levels of reproductive hormones in pinealectomized hamsters kept in short daily photoperiods did not differ significantly from those of pinealectomized animals kept in long days. The results obtained from the present study confirm and extend published observations indicating that, in hamsters that were pinealectomized, darkness or blinding are incapable of inducing atrophy of the reproductive organs or decreasing the levels of circulating reproductive hormones (Hoffman and Reiter, 1965a; Reiter and Hester, 1966; Reiter, 1969; Demaine et al., 1983; Jackson et al., 1984).

These results suggest that the pineal gland mediates the effects of short daily photoperiods on the weights of the reproductive organs and levels of circulating reproductive hormones in hamsters. It has been suggested that the mammalian pineal gland is a neuroendocrine transducer; it mediates the transduction of photoperiodic information into a hormonal message (Wurtman and Anton-Tay, 1969).

The results of the present experiment also indicate that the mean weight of each of the reproductive organs in sham-operated hamsters maintained in short days is higher than that observed in intact hamsters maintained in short days in the preceding experiment (Chapter II). In fact, two of the sham-operated hamsters maintained in short days had much larger testes and accessory sex glands than did the other hamsters subjected to a similar treatment. This may have resulted from partial damage of the pineal gland or its innervation, which may have occurred during sham operation. Several reports indicated that the mammalian pineal gland receives environmental photic information from the eyes through a multisynaptic pathway terminating with postganglionic sympathetic axons to the pineal gland (Moore and Klein, 1974; Moore, 1978; Klein and Moore, 1979). Surgical interruption of the nervi conarii (which innervate the pineal gland) or extirpation of the superior cervical ganglia (which contain the cell bodies of the postganglionic sympathetic fibers that innervate the pineal gland) has been shown to prevent the antireproductive effects of short photoperiods (Reiter and Hester, 1966; Reiter, 1972). Decentralization of these ganglia or interruption of the multisynaptic pathway have been shown to have the same effects (Reiter and Sorrentino, 1972; Rusak and Morin, 1976; Stetson and Watson-Whitmyre, 1976; Eskes et al., 1984). Thus, not only pinealectomy but also interference with the sympathetic innervation of the pineal suppresses photoperiodic effects on reproductive physiology.

The results obtained from the present study also showed that removal of the pineal gland from hamsters kept in long days had no effect on the weights of the reproductive organs as compared with sham pinealectomized animals kept in the same daily photoperiods. This is also in accord with previous interpretations which suggest that hamsters are functionally or "physiologically pinealectomized" if maintained under long daily photoperiodic conditions; exposure to long days inhibits the pineal influence on reproduction (Reiter, 1971; 1978). Therefore, surgical removal of the pineal gland from such animals has little or no effect on the neuroendocrine-reproductive axis.

Although a number of polypeptides and indoles have been isolated from the pineal gland, melatonin is widely considered to be partially or wholly responsible for the endocrine effects of the pineal gland (Reiter et al., 1976; Tamarkin et al., 1976; Cardinali, 1981; Quay et al., 1982; Reiter, 1986). It has been demonstrated that testicular regression can also be induced in hamsters by daily late afternoon injections of melatonin (Tamarkin et al., 1976). Melatonin treatment has been reported to alter both reproductive organ weights and circulating reproductive hormone levels to the same degree as blinding or short photoperiod (Reiter et al., 1976, 1980; Tamarkin et al., 1977a,b; Goldman et al, 1979; Stetson and Watson-Whitmyre, 1986). Despite experimental results obtained by many research groups, little is known about the actual site(s) of action of pineal constituents within the neuroendocrine-reproductive axis.

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IV- EFFECTS OF PHOTOPERIOD AND PINEALECTOMY ON THE FINE STRUCTURE OF THE VENTRAL AND DORSAL LOBES OF THE PROSTATE

INTRODUCTION

Although the Syrian hamster has been widely used in pineal and reproduction studies, the available information on the effects of the pineal on accessory sex glands are mainly dependent on the changes in the weights of these organs (usually the weight of the seminal vesicles and coagulating glands). The effects of pineal manipulations on the fine structure of these organs have not been reported, except by Lin and Wing (1978), who examined the effects of photoperiod and pinealectomy on the fine structure of the seminal vesicles in the Syrian golden hamster. Therefore, the aim of the present study was to determine the effects of photoperiod and pinealectomy on the fine structure of the ventral and dorsal lobes of the prostate. These two glands were studied for the following reasons: 1) in the previous experiments (Chapter II and III) and in our earlier study (Buzzell et al., 1985b), these glands showed different degrees of sensitivity to these manipulations, and 2) details of the fine structure of these glands in the normal adult hamster have not been reported. Previous light microscopic studies include only consideration of the effects of castration on the ventral lobes (Ortiz, 1953; Schmidt and Visek, 1977), and stereology of the ventral and dorsal lobes in normal adult hamsters (Buzzell, 1985) and in hamsters with castration- and pineal-mediated prostatic regression (Buzzell et al., 1985a). Part of the present study has been published (Toma and Buzzell, 1988).

MATERIALS AND METHODS

Thirty young adult hamsters were used in this study (for supply and care of animals, see Chapter II). They were divided into 3 groups of 10 animals each. Animals in one group were pinealectomized according to the method of Hoffman and Reiter (1965), as outlined in Chapter III, and placed in short-day photoperiods (10h:14h, L:D). Those of the other two groups were untreated and maintained either in the same short-day lighting regime or under long-day photoperiods (14h:10h, L:D). The room temperature was maintained at 22-25 °C. All were maintained in these conditions for 10 weeks before sacrifice.

Each hamster was weighed and anesthetized with Metofane. Blood samples were obtained by cardiac puncture and allowed to clot overnight at 4°C. Serum was obtained by centrifugation at 2500 r.p.m. for ten minutes and stored in two aliquots of 500 μl volume at -70 °C for the radioimmunoassay analysis of testosterone and LH as outlined below. The testes were removed and weighed. The accessory sex glands were exposed and bathed with fixative during dissection. The ventral and dorsal lobes of the prostate were removed, weighed, cut into small pieces (for electron microscopy, tissue samples were cut into pieces of less than 2 mm^3) and fixed in 3% glutaraldehyde and 3% paraformaldehyde in 0.13 M sodium phosphate buffer (pH 7.4) (see Appendix 1) for 24 hr at 4 °C. After fixation, tissues were rinsed in

3 changes (10 min. each) of 0.13 M sodium phosphate buffer, and processed for light and electron microscopy.

For light microscopy, tissue samples were dehydrated in a graded series of ethanols, and this was followed by infiltration and embedding in glycol methacrylate using the JB-4 embedding kit (Polysciences, Inc. Warrington, Pa.) (see Appendix 1). Tissue blocks were embedded in plastic block molding cups. The polymerization was allowed to proceed at room temperature overnight.

Sections of 2 μm thickness were cut with a glass knife on a Sorvall Porter-Blum JB-4A microtome. They were flattened by floating on water, and then transferred to a precleaned glass microscope slide. Excess water was removed and the slide was dried on a slide warmer at approximately 45 °C.

The sections were stained with Lee's methylene blue-basic fuchsin stain (Bennett et al., 1976) (see Appendix 1). Slides bearing sections were covered with a few drops of staining solution for 10-15 seconds. They were then rinsed with deionized water, dipped briefly into 50% ethanol for the removal of excess stain, and air dried. The dried slides were mounted under a cover slip using Permount mounting medium (Fisher Scientific Co.), and examined with a Zeiss light microscope. Light micrographs were obtained using 35 mm Kodak Panatomic-X film.

For electron microscopy, tissue samples were postfixed in 1% osmium tetroxide buffered in 0.13 M sodium phosphate buffer (pH 7.4) for 2 hr, rinsed in 3 changes of 0.13 M sodium phosphate buffer, and dehydrated by a graded series of ethanols (see Appendix 1). During dehydration with ethanol, the tissue samples were stained *en block* with 2% uranyl acetate in 80% ethanol for 15 minutes. The final dehydration was carried out in alcohol followed by three changes of 100% propylene oxide to remove the alcohol and prepare the tissue for infiltration with the embedding medium.

Subsequent to pure propylene oxide treatment, the samples were infiltrated at room temperature with a 1:1 propylene oxide:Araldite mixture (Marivac Limited, Halifax, N.S.), followed by a 1:3 propylene oxide:Araldite mixture (see Appendix 1). The final infiltration was accomplished using a pure Araldite mixture, in which tissues were left overnight at room temperature.

The tissue was embedded in fresh Araldite mixture using flat rectangular embedding molds; they were polymerized overnight in a 45 °C oven and then overnight at 70 °C.

For light microscopic observations, and in order to check for proper tissue orientation and content, Araldite sections 0.5-1 μm thick were cut with a glass knife on a Sorvall MT-2 ultramicrotome. The sections were placed on a drop of deionized water on a clean glass slide, and this was placed on a hot plate set at approximately 200 °C. The water was allowed to evaporate and the sections were dried. The sections were stained with 0.5% toluidine blue made in a 1% sodium borate solution. They were covered with several drops of the stain, and the slide was heated until the stain began to steam and a metallic green sheen appeared over the surface of the stain. The slides were rinsed with deionized water, air dried, and a coverslip was mounted.

Ultrathin sections, approximately 60-70 nm thick (silver-gold) were cut with a diamond knife on a Sorvall MT-2 ultramicrotome. The sections were collected on uncoated 300 mesh copper grids, stained on drops of saturated aqueous uranyl acetate for 20-30 minutes, and with Reynold's lead citrate (Reynolds, 1963) for 8 minutes (see Appendix 1). The sections were viewed in a Philips EM-410 at 80 KV. Electron micrographs were obtained using 35 mm Kodak Eastman Fine Grain Release Positive Film 5302.

Radioimmunoassay

Serum levels of testosterone were determined using Coat-A-Count radioimmunoassay kit supplied by the Diagnostic Product Corporation, Los Angeles, CA., with human serum-based standards. The radioimmunoassay for LH was done in the laboratory of Dr. G. Bourne, Department of Physiology, University of Alberta, Edmonton. The reagents for the assay were supplied by the National Institute of Arthrites, Diabetes and Digestive and Kidney Diseases (NIADDK), with rat LH-RP-2 reference preparation as standard.

Statistics

The results of the weights of the accessory sex glands and serum reproductive hormone levels are expressed as mean ± standard error of the mean. The groups were first compared with analysis of variance. Where significant differences were indicated, Student's t-test was then applied to determine groups contributing to these differences. Significant differences between groups were accepted with $P < 0.05$.

RESULTS

General Considerations

Exposure of intact adult Syrian hamsters to short-day photoperiods resulted in a significant reduction in the weights of the testes and dorsal lobes of the prostate, compared to those of animals kept in long days, or pinealectomized ones kept in short days. On the other hand, the weights of the ventral lobes of the prostate in intact hamsters kept in short days did not differ significantly from those of animals kept in long days or pinealectomized ones kept in short days. Circulating levels of LH and testosterone were also significantly reduced in animals kept in short days when compared with pinealectomized hamsters kept in the same photoperiods or intact animals kept in long days (Table IV-1).

Gross Anatomy and Fine Structure

The ventral lobes of the prostate consisted of a pair of small clearly separate lobes, each of which was embedded in fat and lay adjacent to the ventrolateral surface of the urethra and the neck of the bladder. Each emptied by three main ducts into the urethra. The dorsal lobes of the prostate consisted of two large nearly fused lobes that lay on the dorsal surface of the urethra. Each emptied into the urethra via several main ducts.

1- Ventral lobes of the prostate:

A- Long photoperiods:

The secretory components of each ventral prostatic lobe were organized as branched tubular acini. The acini were separated from each other by loose interacinar connective tissue (Fig. IV-1). The lumen of the acinus contained secretory product and was lined with tall columnar secretory cells which were characterized at the light microscopic level by a basally located nucleus and a large clear Golgi area between the nucleus and the luminal border. Occasional basal cells were also found in the epithelium. Both types of epithelial cells were supported by a thin lamina propria (Fig. IV-2). Epithelium plus lamina propria made up a mucosa which was often thrown into branching folds (Fig. IV-1). The acinus was bounded by a muscular stroma, composed of spirally arranged smooth muscle fibers and connective tissue elements (Fig. IV-2).

Numerous microvilli were present at the luminal surface of secretory epithelial cells (Figs. IV-3, 4, 11). Junctional complexes, desmosomes, and interdigitations were found between adjacent cells (Figs. IV-4, 6). The basal plasmalemma was usually relatively straight, but folding of this surface occasionally occurred (Fig. IV-5). Micropinocytotic vesicles were often present at the basal surface (Fig. IV-7).

Profiles of the nuclei of secretory cells were usually round or else elongated in the same axis as the cell. Deep surface indentations of the nuclei were common. Nuclei were usually vesicular with one or more eccentrically located nucleoli and peripheral clumps of heterochromatin (Fig. IV-3).

Rough endoplasmic reticulum (RER) was present throughout the cytoplasm, except for the Golgi area (Fig. IV-3). The RER often exhibited concentric membranous profiles, lamellated parallel profiles, and cisternae with irregular configurations. Concentric profiles usually occupied a supranuclear position (Figs. IV-3, 8), whereas lamellated parallel profiles were found along the lateral plasmalemma (Fig. IV-9). Some secretory cells showed relatively small dilated cisternae of RER, particularly in the apical portion of the cell (Fig. IV-9).

The Golgi complex was very extensive and was often the most obvious feature in the supranuclear portion of the cell (Fig. IV-3). It was composed of highly dilated membranous sacs, small vesicles, and vacuoles of various sizes (Figs. IV-3, 10). Some of the vacuoles contained finely granular material (Fig. IV-10).

Membrane-bounded secretory granules of various sizes and densities were often abundant in the Golgi and apical areas of secretory cells (Figs. IV-3, 9-12). The contents of these granules were homogeneous in some cases, and they often contained flocculent materials of high electron-density (Figs. IV-9-12). Secretory granules appeared to be formed by the large condensing vacuoles arising from the Golgi complex (Fig. IV-10). In the apical cytoplasm, two or more secretory vesicles occasionally coalesced, forming larger units with irregular outlines (Figs. IV-11, 12). The secretion of the granules from the cells into the acinar lumen appeared to take place by exocytosis (Figs. IV-11, 12). Dense material similar to that within the secretory granules was often present within the lumen (Fig. IV-11).

Lysosome-like dense bodies were sometimes present in the Golgi, supranuclear, and occasionally in the basal areas of the secretory epithelial cells (Fig. IV-3). In some cells, these dense bodies were surrounded wholly or partially by cisternae of RER. Mitochondria with lamellated cristae were dispersed throughout the cytoplasm of the secretory cells. They were generally elongated, but round and oval ones were also present (Figs. IV-9, 10).

The basal cells, which were generally flattened or triangular in shape, were located between the basal portions of adjacent secretory epithelial cells. Their lateral and apical plasmalemmae often interdigitated with those of adjacent secretory cells, but their basal surface was relatively straight (Fig. IV-13). Pinocytotic vesicles were occasionally present along the basal plasmalemma. The nuclei of basal cells usually had a triangular or elongated profile and contained an abundance of dense chromatin; lobulated nuclei with invaginations of the nuclear surface were also present. The cytoplasm of basal cells was more electron-dense than was that of the secretory cells and contained fewer organelles. Small cisternae of RER, some free ribosomes, and a few mitochondria were present in these cells.

Both secretory and basal cells were separated from the underlying lamina propria by a thin basal lamina which followed the contours of the basal plasmalemma (Figs. IV-5, 7, 13). The lamina propria was composed of connective tissue, nerves, and capillaries, and also contained fibroblasts, macrophages, plasma cells, vascular pericytes, and occasional mast cells.

The muscular stroma consisted of 4-6 layers of spirally oriented smooth muscle fibers, each of which was surrounded by an external lamina and by connective tissue continuous with that of the lamina propria and interacinar tissue (Figs. IV-2, 13, 14).

The interacinar connective tissue contained the same elements found in the lamina propria. In addition, larger blood vessels, nerves, and fat cells were common. Unmyelinated nerve fibers were common in the lamina propria, among the smooth muscle cells, and in the interacinar connective tissue. However, nerves containing both myelinated and unmyelinated axons were occasionally seen in the interacinar connective tissue close to the outermost layer of the muscular stroma (Fig. IV-14).

B- Short photoperiods:

The ventral lobes of the prostate showed considerable variation in their response to short days exposure. In most acini, the secretory epithelial cells were tall columnar and their cytoplasmic organelles were comparable to those found in animals kept in long photoperiods. The RER in these cells was abundant throughout the cytoplasm, except for the Golgi area (Figs. IV-15, 16). The Golgi complex was extensive. Several large condensing vacuoles were associated with the Golgi complex (Fig. IV-15). Membrane bound secretory granules were present in the apical cytoplasm (Fig. IV-15), and exocytosis of the secretory granules was apparent (Fig. IV-17).

In some acini, there were secretory cells which showed signs of atrophy. Some of these cells, while columnar, were lower and squatter than those found in animals maintained in long days (Figs. IV-18, 19), and often showed a general reduction in the amount of cytoplasm in the infranuclear region of the cell.

The most obvious changes which occurred in the ventral prostate of hamsters exposed to short days were a partial collapse of the cisternae of RER, a moderate reduction in the Golgi complex, and an increase in the number of dense bodies.

In most of the atrophied cells of the ventral prostate, the concentric lamellated cisternae of RER in the supranuclear area, and the vesicular profiles in the apical cytoplasm had largely disappeared (Figs. IV-18-20). The Golgi complex in these cells was generally smaller than that found in intact animals kept in long days. It consisted of short stacked cisternae (Figs. IV-18-20), and the large dilated membranous cisternae had largely disappeared. In the Golgi area, the large condensing vacuoles seen in the epithelium of animals maintained in long days were often not evident.

Secretory granules were often found concentrated in the apical cytoplasm (Figs. IV-18-20). Some of the cells showed fewer secretory granules in the apical cytoplasm than did other cells (Fig. IV-21).

Mitochondria were scattered throughout the cytoplasm. No morphological changes were detected in this organelle, although some of the atrophied cells contained fewer mitochondria than normally seen in intact animals kept in long days.

The nuclei had an almost normal appearance; however, nuclei with large clumps of chromatin in the periphery were occasionally present (Figs. IV-22, 23).

Electron-dense bodies, which were occasionally seen in the secretory cells of animals maintained in long days, were more abundant in the supranuclear and basal portions of the cells (Figs. IV-22, 23). The dense bodies varied in size and shape, and appeared to be predominantly located in the basal cytoplasm (Fig. IV-23).

Occasionally, these bodies contained a large number of small vesicles (Fig. IV-24). Some of the secretory cells showed signs of cytoplasmic dissolution in the central portion of the RER-whorls, forming a large pool of amorphous material (Fig. IV-24).

In pinealectomized hamsters kept in short days, the secretory epithelial cells were similar to those seen in intact animals kept in long days (Fig. IV-25). The cells showed an abundance of RER which occupied most of the cytoplasm except the Golgi area. The RER was composed of numerous lamellated cisternae and associated ribosomes (Figs. IV-26, 27). The concentric lamellated profiles of RER were often present in the supranuclear area (Fig. IV-26); lamellated parallel profiles were found along the lateral plasmalemma (Fig. IV-28), whereas vesicular profiles were often seen in the apical cytoplasm (Fig. IV-28).

The Golgi complex was highly developed, and consisted of stacks of dilated parallel cisternae, vesicles and large vacuoles containing granular material (Fig. IV-25). Secretory granules were present close to the Golgi complex, and in the apical portion of the cell (Fig. IV-28).

2- Dorsal lobes of the prostate:

A- Long photoperiods:

The general architecture of the dorsal lobes of the prostate was similar to that of the ventral prostate (Fig. IV-29). However, some of the secretory acini in the dorsal prostate contained numerous mucosal folds; others were wider and usually showed a flattened mucosa with only occasional short primary mucosal folds. Transitional forms between these types of acini were also present. The mucosa in all types of acini was composed of a simple columnar secretory epithelium with occasional basal cells, supported by a thin lamina propria. Generally, tall columnar cells were present in the acini with numerous mucosal folds (Fig. IV-30), whereas short columnar cells were present in the distended acini with flattened mucosae (Fig. IV-31). In both cases, the secretory cells were characterized at the light microscopical level by centrally or basally located nuclei, and cytoplasm which often exhibited pools of clear amorphous material. All types of acini were bounded externally by a thick muscular stroma (Figs. IV-30, 31).

The luminal surfaces of the secretory cells were sometimes flat with numerous small microvilli (Fig. IV-32), but bulging of the apical surface into the lumen was common (Figs. IV-32, 33). In the short columnar cells of distended tubules, these apical bulges often contained free ribosomes, dilated cisternae of RER, and occasional lysosome-like dense bodies (Figs. IV-32, 34). The apical surface surrounding these bulges was often smooth with a few short microvilli present at the base of the bulge. Some of the bulges showed constrictions at their bases; here the apical rim of the bulge usually contained homogeneous or granular cytoplasm (Fig. IV-32). On the other hand, in the tall columnar cells of the folded tubules, the bulges usually had a smooth surface and contained amorphous or flocculent material (Fig. IV-33). Blebs, which are apical extensions attached to the apical surfaces of the cells by a narrow stalk, were also common. The blebs varied in their size, shape and contents.

Blebs were usually regular in shape, but some with irregular outlines were also present (Figs. IV-36, 37). Some large blebs contained homogeneous or slightly granular material (Fig. IV-36); others contained free ribosomes, dilated cisternae of RER (Fig. IV-37), and multivesicular bodies (Fig. IV-39). Some small blebs contained a single dense body surrounded by a thin rim of cytoplasmic matrix (Fig. IV-38).

Bodies similar to detached blebs often lay free within the lumen of the tubule. These bodies were usually bounded by an intact membrane and contained amorphous homogeneous or slightly granular material. However, free bodies containing cytoplasmic matrix and intact organelles were also present (Fig. IV-39). Some free bodies with mottled contents showed a defect in the surrounding membrane and occasionally appeared to be releasing their contents into the acinar lumen (Fig. IV-35).

Surface microvilli varied in their length, diameter and shape and some branched to form secondary processes whereas others had a vesicular dilation at the distal end (Fig. IV-40).

The lateral cell membranes of adjacent secretory cells were joined near the apical surface by junctional complexes. Desmosomes and interdigitations between cells were common. The basal surface often presented micropinocytotic vesicles and infolding of the plasmalemma.

The nucleus of secretory cells was variable in shape and location. Very often, the short columnar cells contained basally or centrally located nuclei with indentations, and the tall columnar cells contained basally located round or oval nuclei with regular outlines. Both types of cells had eccentric nucleoli and peripherally located heterochromatin (Figs. IV-32, 33).

A striking feature of the secretory epithelial cells of the dorsal prostate was the extraordinarily developed and dilated cisternae of RER. Large dilated cisternae with irregular outlines were common in the basal portion of the low columnar cells from distended acini (Fig. IV-32). The tall columnar cells from folded acini often showed smaller dilated cisternae with regular outlines, which were distributed throughout the cytoplasm (Fig. IV-33). However, there were many transitional forms of RER between the low and tall columnar cells. All types of cisternae of RER were filled with finely granular material.

The Golgi complex was located between the nucleus and the apical border of the cell (Figs. IV-32, 33). It was generally moderately developed, consisting of several parallel stacks of cisternae, secretory vesicles, and vacuoles containing finely granular material (Fig. IV-41). In some cells, the Golgi complex was poorly developed and consisted of only a few short stacked cisternae (Fig. IV-42).

Membrane-bounded secretory granules were often not present. When present, they consisted of dense cores surrounded by electron-translucent material and were found in the Golgi and apical areas (Figs. IV-41, 43). The secretory granules appeared to be discharged into the acinar lumen by exocytosis (Fig. IV-44).

Primary and secondary lysosomes and lysosome-like dense bodies were common in the secretory cells. Oval or elongated mitochondria with lamellated cristae, and coated vesicles and microtubules were often present distributed throughout the cytoplasm. Bundles of microfilaments sometimes lay parallel to the apical plasmalemma.

Triangular or hemispherical basal cells were located between adjacent secretory cells. Sometimes their cytoplasm extended for a distance between the basal portions of adjacent secretory cells and the basal lamina (Fig. IV-45). The nuclei of basal cells were often triangular in profile, but they were occasionally deeply fissured or even lobated. The nucleus was characterized by an eccentrically located nucleolus and peripherally located heterochromatin. Rough endoplasmic reticulum, free ribosomes, and mitochondria were the most common organelles found in these cells.

The secretory cells and the basal cells rested on a thin basal lamina (Figs. IV-32, 33, 45). The lamina propria contained connective tissue, unmyelinated nerves, and capillaries. Macrophages, fibroblasts, plasma cells and occasional mast cells were also present in the lamina propria.

The muscular stroma consisted of several layers of spirally arranged smooth muscle fibers, each of which was surrounded by an external lamina and connective tissue. The interacinar connective tissue contained large blood vessels, autonomic ganglia, and nerves. Both unmyelinated, and mixed nerves containing myelinated and unmyelinated axons were present.

B- Short photoperiods:

The major changes in the secretory epithelial cells of the dorsal lobes in short-day photoperiods consisted of a partial collapse or an obliteration of the normally highly dilated cisternae of RER. Both types of secretory epithelial cells, the short columnar cells of the distended acini (Fig. IV-46) and the tall columnar cells of the folded acini (Fig. IV-47), showed a marked reduction in the amount of RER. In some secretory epithelial cells which contained dilated cisternae of RER, there appeared to be fewer ribosomes on the membrane, compared with those observed in long days (Fig. IV-49).

In short columnar cells, the highly dilated and confluent cisternae of RER, which were normally seen in the basal and perinuclear regions of the cytoplasm, were completely obliterated in some cells (Fig. IV-46) or persisted as small dilated cisternae in others. The secretory cells of the folded acini, on the other hand, generally showed few and small vesicular cisternae (Figs. IV-48, 50) or fragments of flattened cisternae of RER scattered throughout the cytoplasm (Figs. IV-47, 51).

The Golgi complex was usually small (Figs. IV-46, 51). Secretion granules seemed to be totally absent in the Golgi region (Figs. IV-46, 51). However, in some of the secretory cells of the folded acini, numerous vacuoles and vesicles, some of which contained electron-dense granules similar to those found in the Golgi area of intact animals kept in long days, accumulated in the apical cytoplasm (Figs. IV-47, 48). Most of these vacuoles and vesicles were in close apposition to the luminal plasma membrane.

The nuclei were either oval (Fig. IV-48) or exhibited indentations in their surfaces (Fig. IV-46); crenated nuclei were also present (Fig. IV-47). Clumps of heterochromatin were often seen in the peripheral region of the nuclei. Mitochondria were found dispersed within the cells. The number of lysosomes increased with short days exposure (Fig. IV-51). Dense bodies containing membranous formations, and large autophagic vacuoles containing large electron-dense bodies and digested material were occasionally encountered (Figs. IV-52, 53).

At the luminal surface, microvilli were barely detectable in some cells (Figs. IV-49, 50), but still present in others. A decrease in the formation of apical bulges and blebs was evident. In many cells exhibiting a marked involution of the RER system, the basal plasma membrane was highly folded, and this was externally lined by the basal lamina (Figs. IV-48, 54).

In pinealectomized hamsters maintained in short days, the cytoplasm of the secretory cells in the distended and folded acini contained numerous dilated cisternae of RER (Figs. IV-55-58). The Golgi complex in some of the secretory cells in pinealectomized hamsters was better developed than that generally found in intact animals kept in long or short photoperiods (Figs. IV-57, 58). In addition, these cells contained a number of secretory vesicles with eccentrically located dense granules in Golgi and apical regions of the cell (Figs. IV-57-59). Extrusion of secretory granules into the acinar lumen appeared to take place by exocytosis (Fig. IV-60).

The nuclei of the secretory cells in the pinealectomized hamsters were comparable to those of animals maintained in long days.

The luminal surface of the secretory cells in pinealectomized hamsters was covered with numerous microvilli. Apical bulges and blebs of various sizes, configurations and contents were regularly encountered (Figs. IV-55, 56).

DISCUSSION

Although a general histological pattern existed for the ventral and dorsal prostatic lobes in Syrian hamsters, cytological differences were present between the secretory epithelial cells of these lobes. Each lobe of the prostate consisted of tubular acini draining into the urethra. The acini were lined by a mucosa consisting of a simple columnar secretory epithelium with occasional basal cells, supported by a thin lamina propria. This mucosa was often thrown into branching folds. The acini were bounded externally by a prominent muscular stroma and separated one from another by loose connective tissue, provided with blood vessels and nerves.

In the ventral lobes of hamsters kept in long days, the secretory epithelial cells were characterized by the presence of microvilli on the luminal surface, a well developed RER with flattened cisternae, a large Golgi complex, and membrane-bounded secretory granules of various sizes and densities. The secretory epithelial cells of the dorsal lobes, on the other hand, were characterized by the presence of apical projections of various sorts, highly dilated cisternae of rough endoplasmic reticulum, and a moderately developed Golgi complex.

The secretory epithelial cells of the ventral lobes of hamsters kept in long days have features in common with secretory cells of the ventral lobes of the prostate of the mouse and rat (Brandes and

Portela, 1960; DiDio, 1971). However, the Golgi complex was more extensive than it is in these other species, consisting of dilated sacs occupying a large portion of the cell. Furthermore, the secretory granules often contained flocculent electron-dense material which has not been previously described in other accessory sex glands. The nature of this material is unknown.

In the dorsal lobe of the hamster prostate, some acini had many mucosal folds and were lined with tall columnar secretory cells, whereas others were distended and were lined with short columnar secretory cells. Sugimura et al. (1986) demonstrated in the ventral and dorsolateral lobes of the mouse prostate that the distal tips of acini were highly folded whereas the proximal ducts contained few mucosal folds. Thus, the differences in the degree of mucosal foldings among the acini of the dorsal prostate of hamster may represent different regions within the secretory units, and/or could be due to the degree of distension by the secretory material within the acinar lumen.

In the dorsal prostate of hamsters maintained in long days, small dilated cisternae of RER, present as isolated profiles throughout the cytoplasm, were common in the tall columnar cells from folded acini. Most of the short columnar secretory cells from distended acini contained highly dilated irregular profiles of RER occupying most of the basal and perinuclear areas. Ultrastructural differences between these types of secretory cells may reflect differences in their physiological activity.

The distribution and dilation of RER in the short columnar cells were similar to that reported for the secretory cells of the dorsal prostate of the rat (Brandes and Groth, 1961). Brandes and Groth (1961) also demonstrated another type of secretory cell in the dorsal prostate of the rat. Here the rough endoplasmic reticulum appeared as a diffuse, relatively poorly defined continuous phase, and the cytoplasm as small isolated profiles within the rough endoplasmic reticulum cisternae. In the present study, this type of secretory cell was not encountered.

The marked development of the RER in the secretory cells of the ventral and dorsal lobes of the hamster prostate indicates that both of these glands are involved in synthesis and secretion of proteinaceous material. In the ventral lobe, this material is probably transported to the Golgi stacks which package the secretory products into condensing vacuoles, and these eventually form mature secretory granules which are discharged into the acinar lumen by exocytosis. This pathway of the secretory material has been demonstrated in the ventral lobe of the rat prostate using [^{3}H]leucine as a marker for secretory protein (Flickinger, 1974).

The extremely dilated rough endoplasmic reticulum of the secretory cells of the dorsal lobe suggests that these cells also undergo marked synthesis of protein. The Golgi complex in these secretory cells is small, and secretory granules are relatively uncommon. Thus, it is likely that most of the protein produced by the rough endoplasmic reticulum is stored within the dilated cisternae.

Another feature of the secretory cells of the dorsal lobes in hamsters kept in long days was the presence of apical bulges and blebs of various sizes, shapes, and contents. Apical blebs have also been described in secretory cells of accessory sex glands in other rodents and in humans (Aumuller and Adler, 1979; Brandes and Portela, 1960; Nicander et al., 1974; Stone et al, 1977; Wong and Tse, 1981). The presence of apical blebs here suggests that the mechanism of secretion is mainly apocrine. Some authors, however, pointed out that blebs are rarely seen in prostatic tissues fixed by perfusion and feel that these are artifacts due to immersion fixation (Dahl et al., 1973a; Helminen and Ericsson, 1970). They suggested that exocytosis is the major mode of secretion in rat accessory sex glands. On the other hand, Aumuller and Adler (1979), using rat dorsal prostate fixed by perfusion, found that apical blebs were the most common indications of the release of secretory material and supported the concept of apocrine secretion in this gland. In the present study, apical blebs and free bodies resembling released apical blebs were commonly seen in the dorsal lobe of the prostate of the hamster fixed by immersion. Occasional secretory granules and signs of exocytosis were also seen, indicating that both apocrine and merocrine secretion occur in this gland.

Microvilli in the secretory cells of the dorsal prostate of the hamster often showed vesicular dilations at their distal ends. Similar observations have been reported for the coagulating gland of the guinea pig (Wong and Tse, 1981) and the anterior lobe of the prostate gland of the rabbit (Nicander et al., 1974). The presence of such microvilli may represent a form of microapocrine secretion.

In the prostatic epithelium of both the ventral and dorsal lobes, basal cells were present among the secretory cells. They were characterized by a paucity of cytoplasmic organelles and an absence of secretory granules. Sinha and Bentley (1984) have demonstrated that basal cells in the ventral lobes of the prostates of testosterone-treated castrated rats incorporated [^{3}H]thymidine and hypertrophied to become intermediate-type cells which then divided. They suggested that the basal cells are the precursors of secretory epithelial cells. It has also been suggested that basal cells participate in regulating the transport of material between the stroma and the acinar secretory cells, as micropinocytotic vesicles are often present on the basal cell membrane (Ichihara and Pelliniemi, 1975; Kimura and Ichihara, 1980).

Thus, ultrastructural differences exist between the secretory epithelial cells of the ventral and dorsal prostatic lobes in the Syrian hamster. Although the secretory cells in both lobes appear to synthesize protein, the mechanism of secretion in the ventral lobes seems to be exclusively merocrine, whereas that in the dorsal lobes is primarily of the apocrine type, but with an important merocrine component.

The fine structural changes in the secretory epithelial cells of the dorsal prostatic lobes of intact hamsters exposed to short days, were more dramatic than those observed in the ventral lobes. In most of the secretory epithelial cells of the dorsal lobes, indications of cytological secretory activity were depressed or eliminated. The weights of the dorsal lobes in hamsters kept in short days were also significantly lower than those of animals kept in long days, but no significant differences were observed in the weights of the ventral lobes with these treatments.

In hamsters maintained in short-day photoperiods, although some of the secretory cells in the ventral lobes showed signs of atrophy, most of the secretory cells were identical to those observed in animals maintained in long days. The most obvious changes in the atrophied cells were regression of the RER and Golgi complex, and an increase in the number of electron-dense bodies.

In the dorsal lobes of the prostate of hamsters maintained in short days, a large number of the secretory cells exhibited a considerable regression of the RER cisternae, an increase in the number of lysosomes, and a decrease in the number of microvilli, apical bulges and blebs at the luminal surface of the cell. Additionally, the nuclei in some of the tall columnar cells, which are normally oval in shape in animals kept in long days, were indented or crenated.

It is well established that in hamsters, androgen deprivation by castration or exposure to short-day photoperiods leads to a significant reduction in the weights of the accessory sex glands (Gaston and Menaker, 1967; Elliott, 1976; Buzzell et al., 1985b). In both treatments, the ventral prostate usually loses less weight than does the dorsal prostate (Buzzell et al., 1985a,b). This was also evident at the ultrastructural level where exposure of hamsters to short days had little or no effects on the secretory epithelial cells of the ventral lobes.

Schmidt and Visek (1977), who examined the effects of castration on the ventral lobes of the prostate, Cowper's glands, seminal vesicles and coagulating glands in adult hamsters, reported that the ventral prostate showed less response to androgen deprivation than other accessory sex glands. Buzzell et al. (1985a) also noted that short-day photoperiods had relatively little effect upon stereological parameters in the ventral lobes, compared with those observed in the dorsal lobes. The ventral prostate of hamsters maintained in short days showed no significant changes in the proportion of the gland consisting of acinar or interacinar components, compared to those observed in long days. In the dorsal prostate, on the other hand, short-day exposure resulted in an increase in the proportion of the gland made up of interacinar tissue. Furthermore, within the acini of the dorsal prostate in short days, there were decreases in the volume fractions of lumen and large increases in those of lamina propria and muscular stroma (Buzzell et al., 1985a). It is not clear, from these observations, whether short-day exposure resulted in hypertrophy of the interacinar tissue, or whether the interacinar tissue surrounding the collapsed acini was less stretched, compared to that in long days (surrounding large acini filled with secretory material), and this resulted in thicker lamina propria and muscular stroma. In the present study, no apparent changes were observed in the fine structure of the interacinar tissue of the ventral and dorsal lobes of the prostate with different treatments.

The changes observed in the dorsal lobes of the prostate in hamsters with short days exposure, were similar to those reported for the dorsal lobes of the prostate of rats (Dahl and Kjaerheim, 1973) and the coagulating glands of rats and hamsters (Brandes et al., 1962b; Dahl and Tveter, 1973; Cavazos, 1975) after castration. These glands in intact animals are also characterized by a highly dilated cisternae of RER, similar to those present in the dorsal prostate of hamsters. Dahl and Kjaerheim (1973) indicated that, in the dorsal prostate of castrated rats, there was atrophy of the RER with reduction in the number of the RER profiles. Furthermore, blebing of the apical surface was less pronounced than in intact animals.

In short days maintained hamsters, involution of the components of the Golgi complex was evident in some of the secretory cells of the ventral lobes and in most of the secretory cells in the dorsal lobes. In the ventral lobes, the Golgi complex was reduced in some cells to groups of short collapsed cisternae with few condensing vacuoles and vesicles. Secretory granules in these cells tended to be concentrated in the apical cytoplasm. In the dorsal prostate of hamsters maintained in short days, numerous membrane-bound vacuoles, which may represent remnants of secretory granules, accumulated in the apical cytoplasm.

In short days exposed animals, the ultrastructural findings reported here may indicate a decrease in protein synthesis and secretory activity of these glands, relative to those in long days.

Correlative biochemical and ultrastructural studies have shown that postcastration changes in RER are accompanied by a decrease in the protein synthesis capacity of the rat prostatic cells (Brandes and Groth, 1963).

In the ventral prostate of hamsters maintained in short days, there was an increase in the number of electron-dense bodies in the supranuclear and basal regions of the secretory cells. Occasionally dense bodies containing numerous small vacuoles were also present. In addition, some of the secretory cells showed signs of cytoplasmic dissolution in the central portion of RER-whorls, forming a large pool of amorphous material. In the dorsal prostate, the numbers of lysosomes and dense bodies were increased and giant autophagic vacuoles were present. An increase in the number of these structures has also been observed in the prostate gland and coagulating glands of castrated rats (Brandes and Groth, 1963; Brandes et al., 1962a; Dahl and Kjaerheim, 1973; Dahl and Tveter, 1973), and the coagulating glands of aged hamsters and rats (Harkin, 1961; Grandos and Beregi, 1983). It has been demonstrated that the dense bodies represent different forms of lysosomes, since they have the enzymatic activity of lysosomes (Brandes et al., 1962a; Brandes and Groth, 1963). The accumulation of lysosomes and autophagic vacuoles may be related to the involutionary changes occurring in these cells (Brandes et al., 1962a; Paris and Brandes, 1974).

In dorsal prostates of intact hamsters maintained in short days, foldings of the epithelial basal plasma membrane was also noted in some acini. This may reflect a reduction in the volume of the epithelial cells. Foldings of the basal surface membrane have also been reported in the secretory epithelium of the coagulating glands of rats after castration (Brandes et. al., 1962b).

The present ultrastructural study reveals that a general atrophy and involution of cytoplasmic organelles take place after 10 weeks exposure to short days. These features are similar to those reported in ultrastructural studies of the post-castration involution of the accessory sex glands in rodents, such as the ventral, lateral and dorsal lobes of the prostate gland in rat (Brandes, 1966; Brandes and Groth, 1963; Dahl and Kjaerheim, 1973; Dahl and Tveter, 1973), and the coagulating glands of rats and hamsters (Brandes et al., 1962b; Dahl and Tveter, 1973; Cavazos, 1975). It is well established that the structural pattern and the functional properties of the epithelium of the male accessory sex glands are dependent upon androgens. Therefore, they involute after castration (Price and Williams-Ashman, 1961; Brandes, 1966, 1974; Cavazos, 1975) and then regrow to normal structure following androgen administration (Dahl et al., 1973b; English et al., 1985; Sugimura et al., 1986). The decrease in circulating testosterone levels observed in hamsters maintained in short days could therefore be the major factor influencing the structural collapse in the prostate glands.

In the present study, removal of the pineal gland prevented the effects of short days on the fine structural alterations of these glands. The weight of the testes and prostate glands and serum testosterone and LH levels in pinealectomized animals also did not differ significantly from those detected in animals kept in long days. These results indicate that the effects of short days on the fine structure of the secretory epithelial cells of the ventral and dorsal lobes of the prostate are mediated by the pineal gland, probably due to the effects of pineal hormones on the neuroendocrine-gonadal axis, resulting in decreased serum testosterone levels and the subsequent regression of the prostate glands. Nevertheless, pineal secretions may also exert direct effects on the accessory sex glands. Ronnekleiv and McCann (1975) reported that the atrophy of seminal vesicles induced by castration in rats was partially prevented by removal of the pineal gland, suggesting a direct effect of the pineal on the accessory sex glands. Shirama et al. (1981) reported that sham-pinealectomized rats exposed to constant darkness differed from pinealectomized animals by showing lower plasma dihydrotestosterone concentrations and accessory sex organ weights, but no significant differences were found in plasma LH, FSH and testosterone levels or testes weight, suggesting direct effects of pineal secretions on the accessory sex glands.

In the present study, although pinealectomy in short days did not produce marked changes in the glandular epithelium of the prostate, the well developed Golgi complex and the increase in the number of secretory granules in the dorsal prostate may suggest an augmented activity of this gland over that of intact animals in long days. Lin and Wing (1978) also showed that the secretory cells of seminal vesicles of pinealectomized or superior cervical ganglionectomized hamsters exhibited cytological features suggestive of enhanced secretory activity.

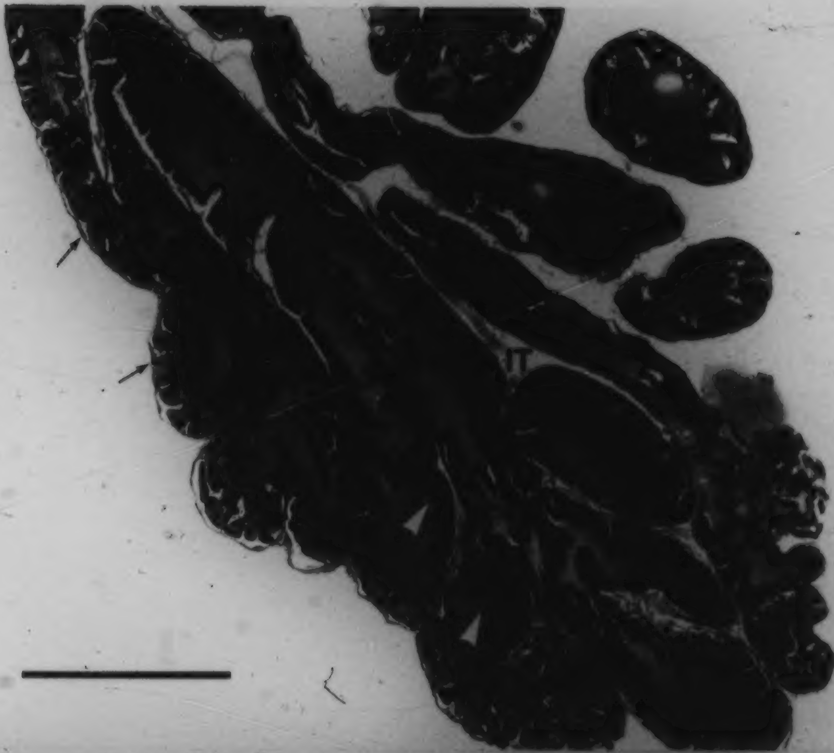
Table IV-1. Weights of testes and ventral and dorsal lobes of the prostate, and serum levels of testosterone and luteinizing hormone (LH) in intact and pinealectomized (Pinx) hamsters after 10 weeks of exposure to respective photoperiods (i.e., 14L:10D, or 10L:14D cycles).

	Intact		Pinx
	14L:10D	10L:14D	10L:14D
Testes (gm)	3.22 ± 0.15	1.86 ± 0.36 *	3.11 ± 0.22
Ventral prostate (mg)	55.70 ± 3.10	51.67 ± 4.62	63.70 ± 5.76
Dorsal prostate (mg)	158.70 ± 10.40	85.00 ± 12.79 *	169.10 ± 21.94
Serum testosterone (ng/ml)	1.18 ± 0.10	0.21 ± 0.08 *	0.71 ± 0.06
Serum LH (ng/ml)	1.69 ± 0.29	0.28 ± 0.15 *	1.12 ± 0.40

Values are mean ± standard error of the mean.
*$P < 0.05$ compared with intact, 14L:10D and Pinx 10L:14D.

Fig. IV-1. Ventral prostate-intact, long days: Survey light micrograph illustrating numerous acini containing abundant secretory material. Note the highly folded mucosa (arrowheads), and the surrounding fibromuscular stroma (arrows). L, acinar lumen; IT, interacinar tissue. Bar equals 0.5 mm.

Fig. IV-2. Ventral prostate-intact, long days: Light micrograph of part of the acinar wall showing acinar lumen (L), epithelium (E), lamina propria (LP) and smooth muscle fibers (SM) of the muscular stroma. The epithelium consists of tall columnar secretory cells, and occasional basal cells (BC). Note the supranuclear light areas in the secretory epithelial cells (arrowhead) which correspond to the Golgi region in Fig. IV-[illegible]. Bar equals 20 μm.



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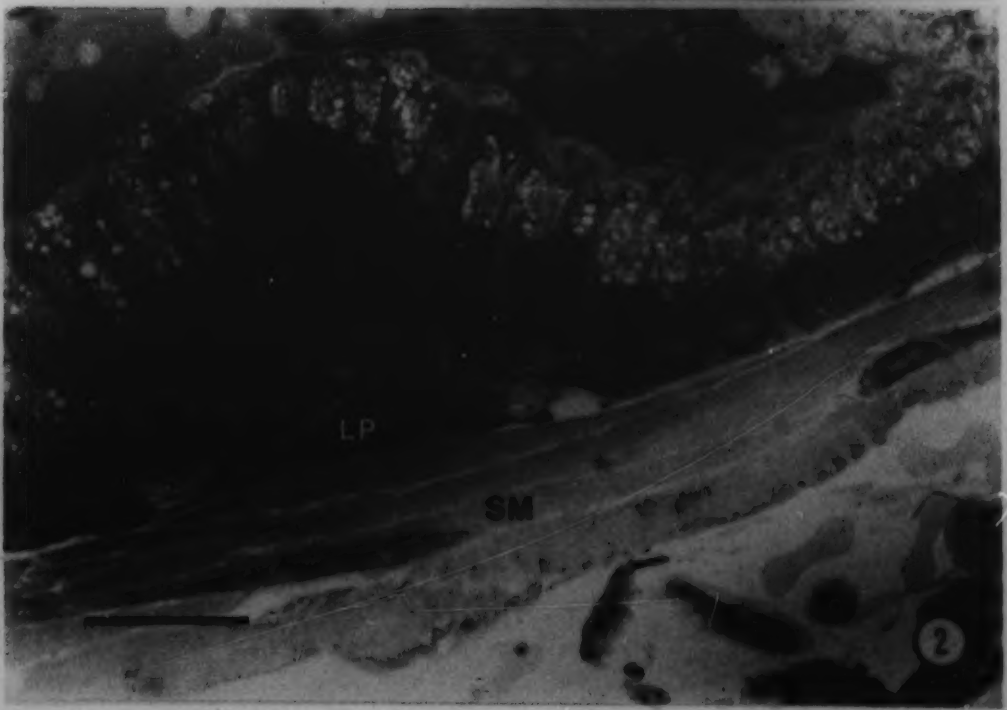

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Fig. IV-3. Ventral prostate-intact, long days: Survey electron micrograph of the columnar secretory cells. The cells are characterized by the basally located nucleus (N), a well-defined system of rough endoplasmic reticulum (RER), a prominent Golgi complex (G), and numerous secretory granules (SG) in the Golgi and apical areas. Dense bodies (DB) are also visible. Microvilli (MV) dominate the luminal surface. Bar equals 3 μm.

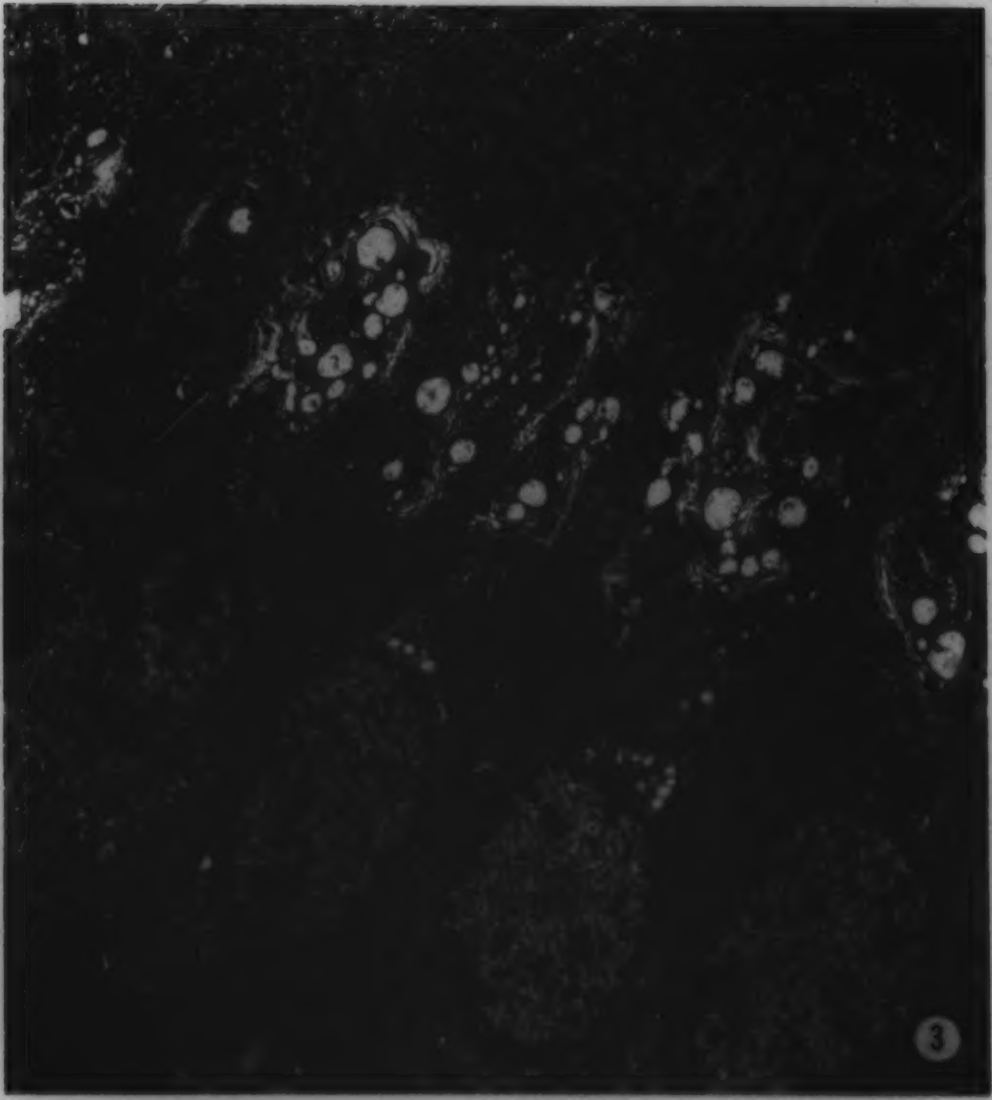

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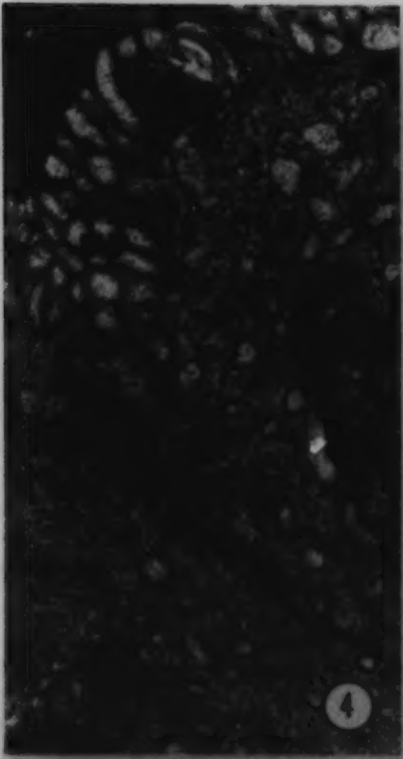
Fig. IV-4. Ventral prostate-intact, long days: Apices of two contiguous secretory cells. The luminal surface is composed of closely packed microvilli (MV). The cells are joined to one another at their apical margins by junctional complexes that include desmosomes (arrows). The apical cytoplasm exhibits vesicular cisternae of rough endoplasmic reticulum (RER), clusters of free ribosomes, mitochondria (M), and secretory granules (SG) which appear to be in the process of exocytosis. Bar equals 0.5 μm.

Fig. IV-5. Ventral prostate-intact, long days: Basal region of the secretory epithelial cells and part of the underlying lamina propria and smooth muscle fibers (SM) of the muscular stroma. The basal cytoplasm contains both lamellar and vesicular cisternae of rough endoplasmic reticulum (RER). The basal plasmalemma presents numerous infoldings along the basal surface of the cells (arrows). Note the bundle of unmyelinated nerve fibers (arrowheads) in the lamina propria. BL, basal lamina; FB, fibroblast. Bar equals 2 μm.

Fig. IV-6. Ventral prostate-intact, long days: The boundary between adjacent secretory cells. The basal portions of the lateral plasmalemmae are extensively interdigitated. Bar equals 1 μm.

Fig. IV-7. Ventral prostate-intact, long days: Basal portion of a secretory epithelial cell. Pinocytotic activity is evident at the basal surface (arrow). BL, basal lamina; LP, lamina propria. Bar equals 1 μm.



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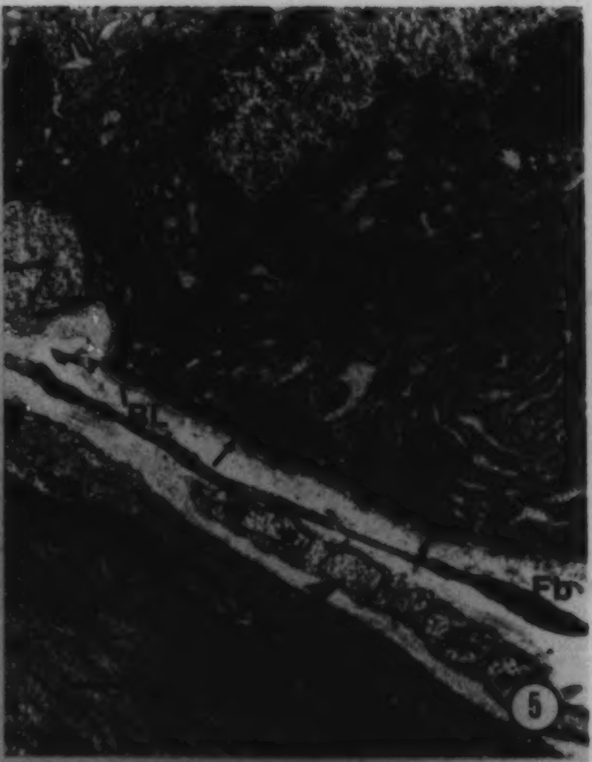

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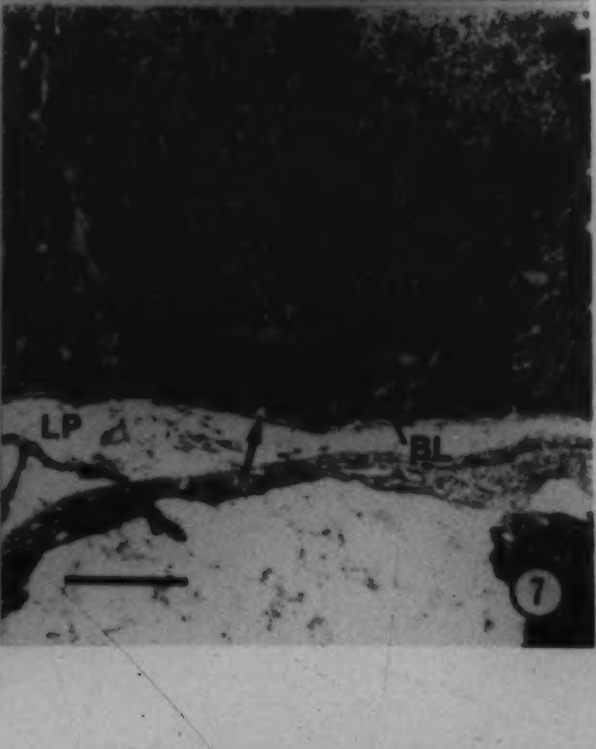

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Fig. IV-8. Ventral prostate-intact, long days: Supranuclear region of a secretory epithelial cell, illustrating concentric and parallel arrays of the lamellated cisternae of rough endoplasmic reticulum (RER). The cisternae contain an opaque, flocculent substance. N, nucleus. Bar equals 0.75 μm.

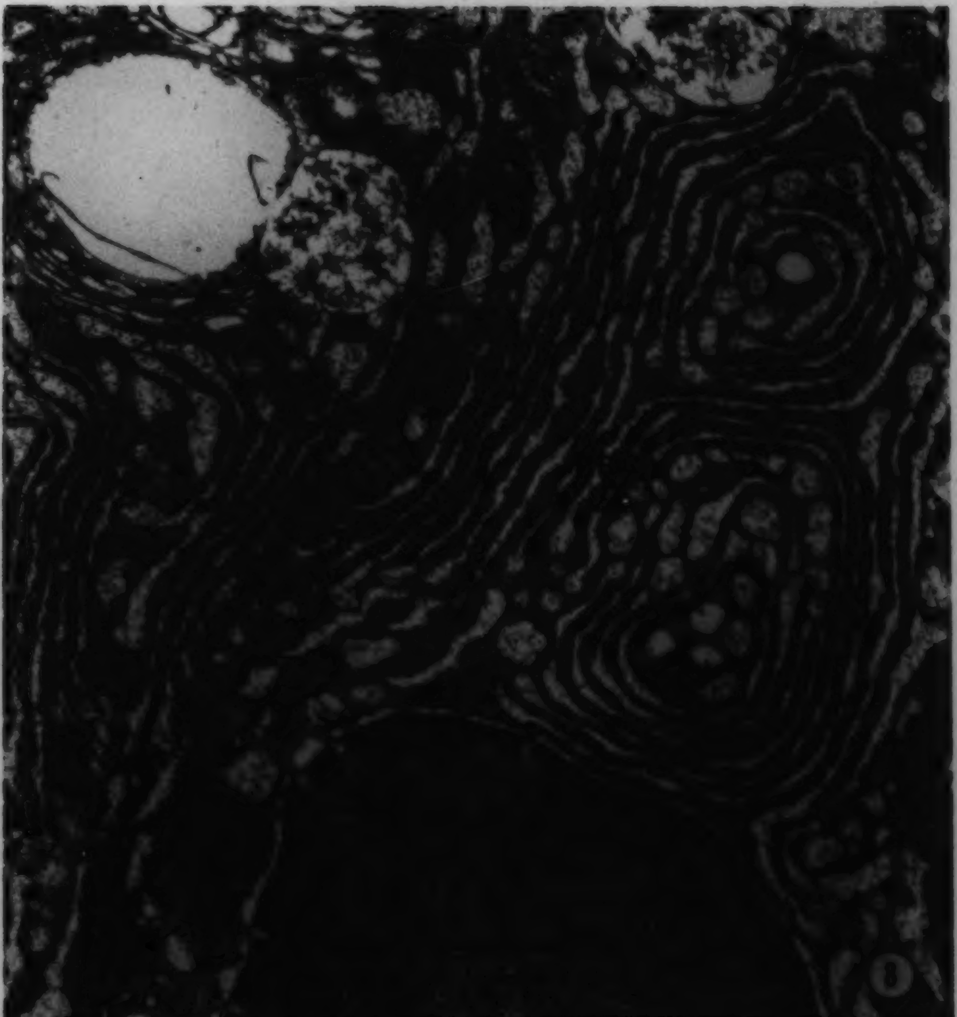
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Fig. IV-9. Ventral prostate-intact long days: Apical-lateral portion of adjacent secretory cells, illustrating the lamellar and vesicular cisternae of rough endoplasmic reticulum (RER). Note the parallel arrays of the cisternae along the lateral plasmalemma. M, mitochondria; SG, secretory granules. Bar equals 1 μm.

9

Fig. IV-10. Ventral prostate-intact, long days: Part of the Golgi area showing various maturation stages of the secretion granules (SG). The Golgi complex (G) is composed of several stacked cisternae. Vacuoles (V) found close to the Golgi complex may be precursors of the secretory granules. Bar equals 1 μm.

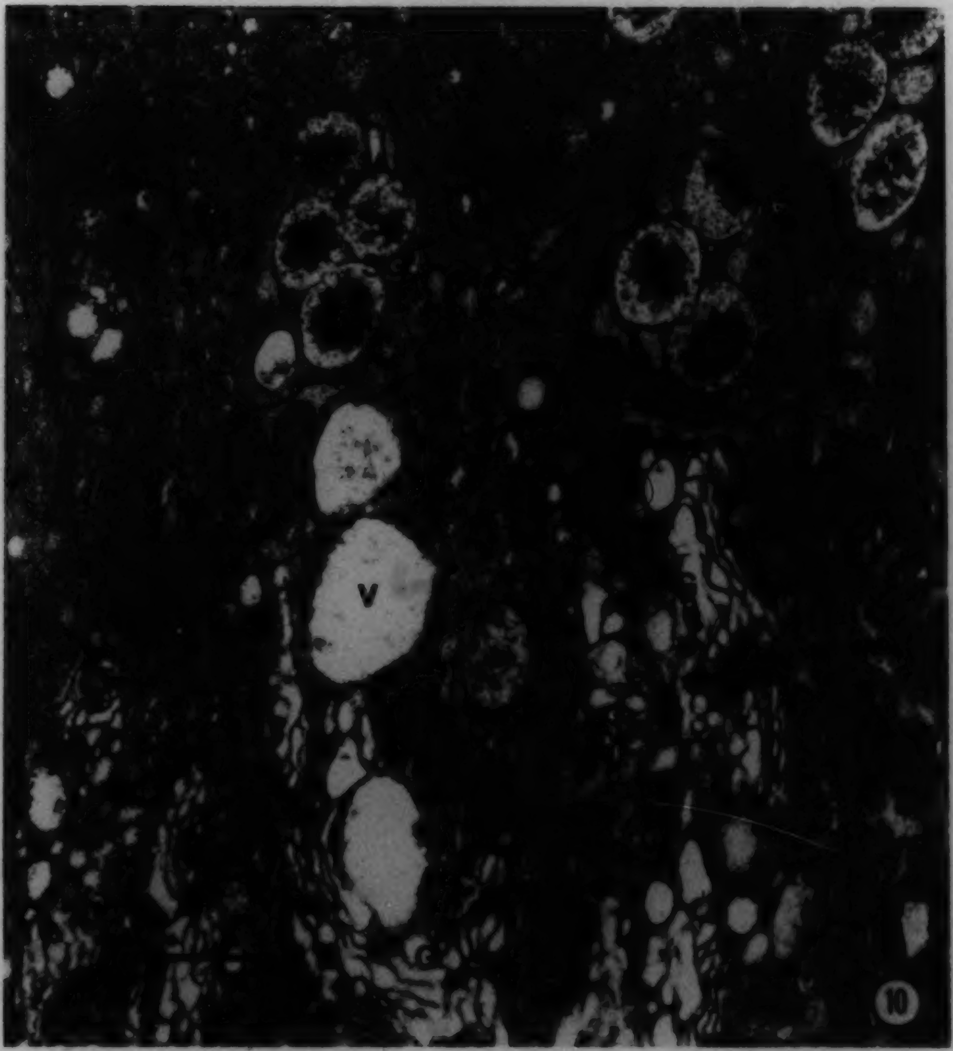
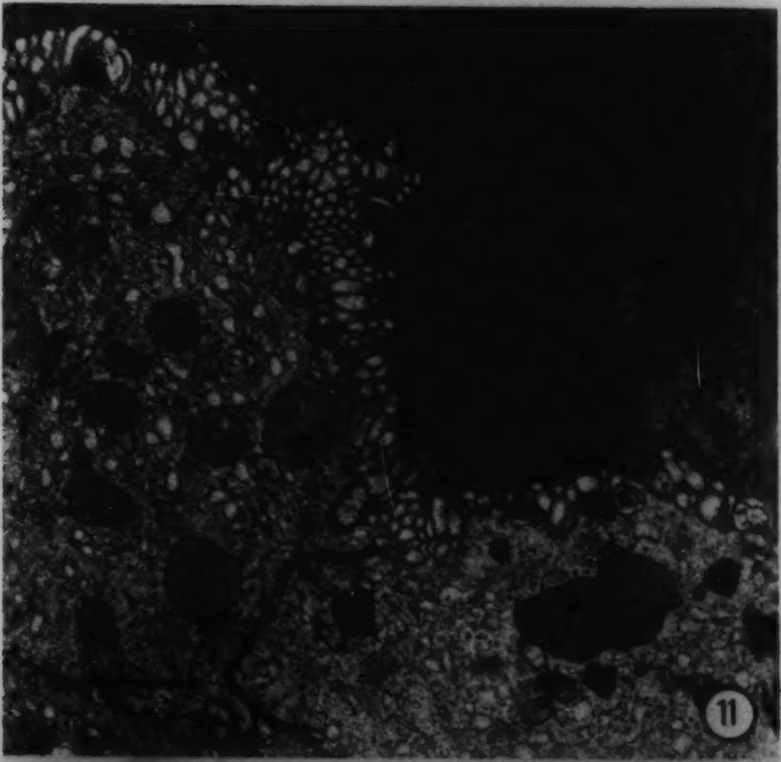

V
G
10

Fig. IV-11. Ventral prostate-intact, long days: Apices of two secretory cells. The apical cytoplasm presents numerous secretory vesicles, containing amorphous, and electron-dense secretory granules (open arrows). Some of these vesicles appear to be in the process of exocytosis. Note the electron dense granules at the bases of the microvilli (arrow), which may represent the newly released secretory material. The acinar lumen (L) contains discharged secretory products with electron-density similar to that found in the secretory vesicles. Bar equals 1 μm.

Fig. IV-12. Ventral prostate-intact, long days: Apical portion of a secretory cell illustrating the confluence of the secretory vesicles, forming a large vesicle containing flocculent materials of high electron-density (arrow). Exocytosis is apparent. Bar equals 0.5 μm.



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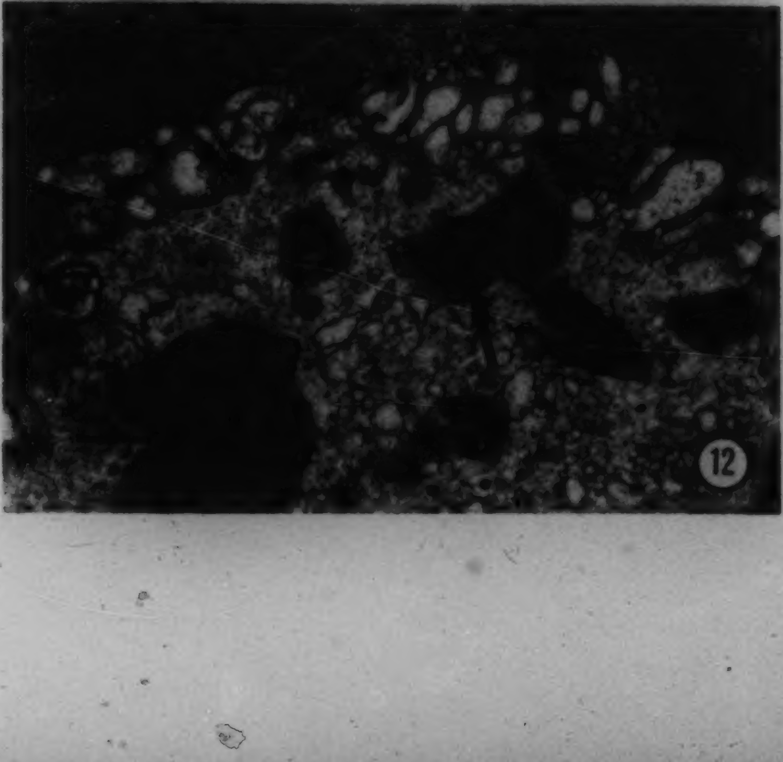

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Fig. IV-13. Ventral prostate-intact, long days: A triangular basal cell interposed between two secretory cells and basal lamina. The cell surfaces of neighbouring basal cell and secretory epithelial cells, are interdigitated (arrows), and also show a desmosome (open arrow). A pinocytotic vesicle is present along the basal plasmalemma (arrowhead). N, nucleus; SM, smooth muscle fibers of the muscular stroma. Bar equals 1 μm.

Fig. IV-14. Ventral prostate-intact, long days: A bundle of myelinated (open arrows) and unmyelinated (arrowhead) nerve axons invested by Schwann cells is present in the interacinar tissue close to the outermost layer of the muscular stroma. SM, smooth muscle fibers. Bar equals 4 μm.

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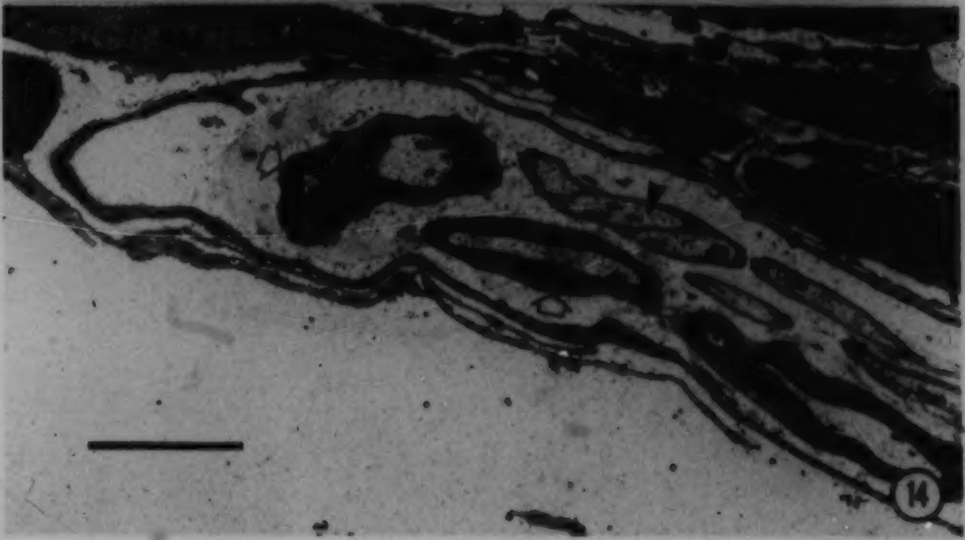

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Fig. IV-15. Ventral prostate-intact, short days: The secretory cells are richly endowed with rough endoplasmic reticulum (RER) and a large Golgi (G) area is present. The apical cytoplasm of one of the secretory cells (upper right corner) contains a number of secretory granules (SG) and small vesicular profiles of rough endoplasmic reticulum (arrowheads). Bar equals 2 μm.

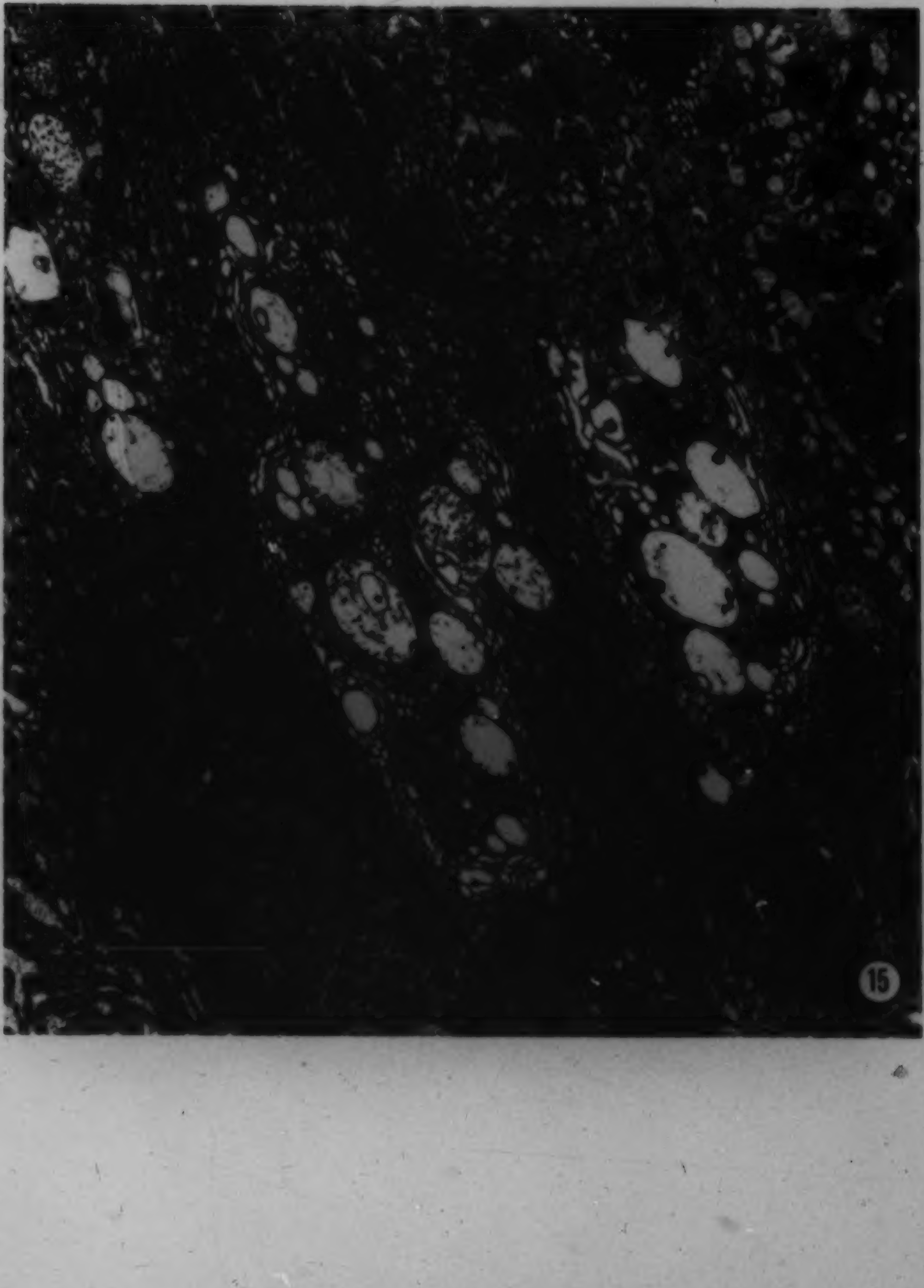
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Fig. IV-16. Ventral prostate-intact, short days: Section through the supranuclear portions of secretory epithelial cells. Note the well developed system of the rough endoplasmic reticulum (RER). The concentric and parallel lamellated cisternae are still present. DB, dense bodies; M, mitochondria. Bar equals 1 μm.

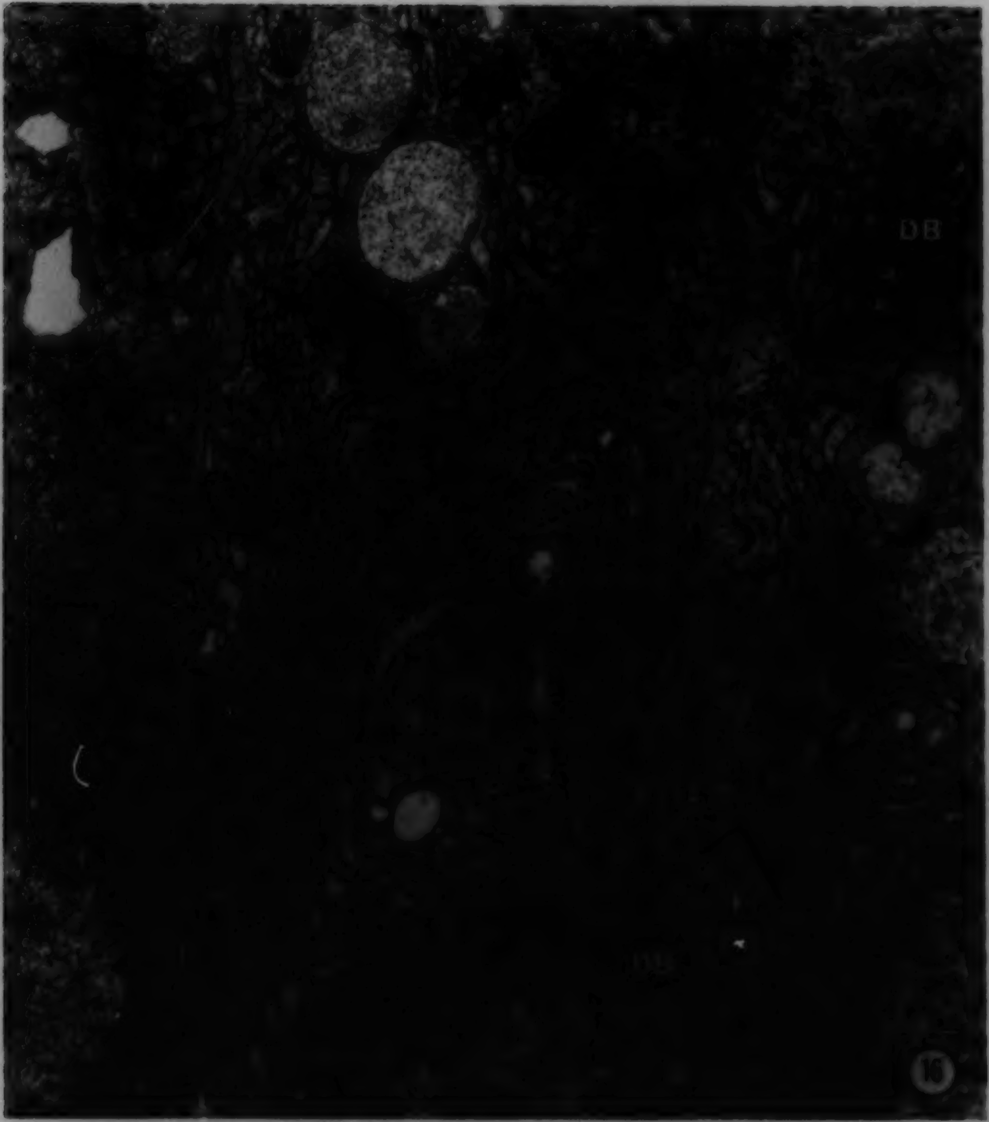
DB
16

Fig. IV-17. Ventral prostate-intact, short days: Section is through the apical portion of the secretory epithelial cells. The apical cytoplasm shows numerous secretory vesicles containing amorphous and electron-dense secretory granules (SG). Note the confluence of the secretory vesicle (arrowheads). Exocytosis is apparent. Open arrows indicate junctional complexes including desmosomes. Bar equals 1 μm.

⑰

Fig. IV-18. Ventral prostate- intact, short days: The rough endoplasmic reticulum (RER) is still present in the perinuclear area, but the concentric lamellated cisternae in the supranuclear area and the parallel lamellated cisternae and vesicular profiles in the apical cytoplasm have largely disappeared. Note the small Golgi complex (G) as compared with that seen in Figs. IV-3 and IV-15. SG, secretory granules; V, condensing vacuoles. Bar equals 2 μm.

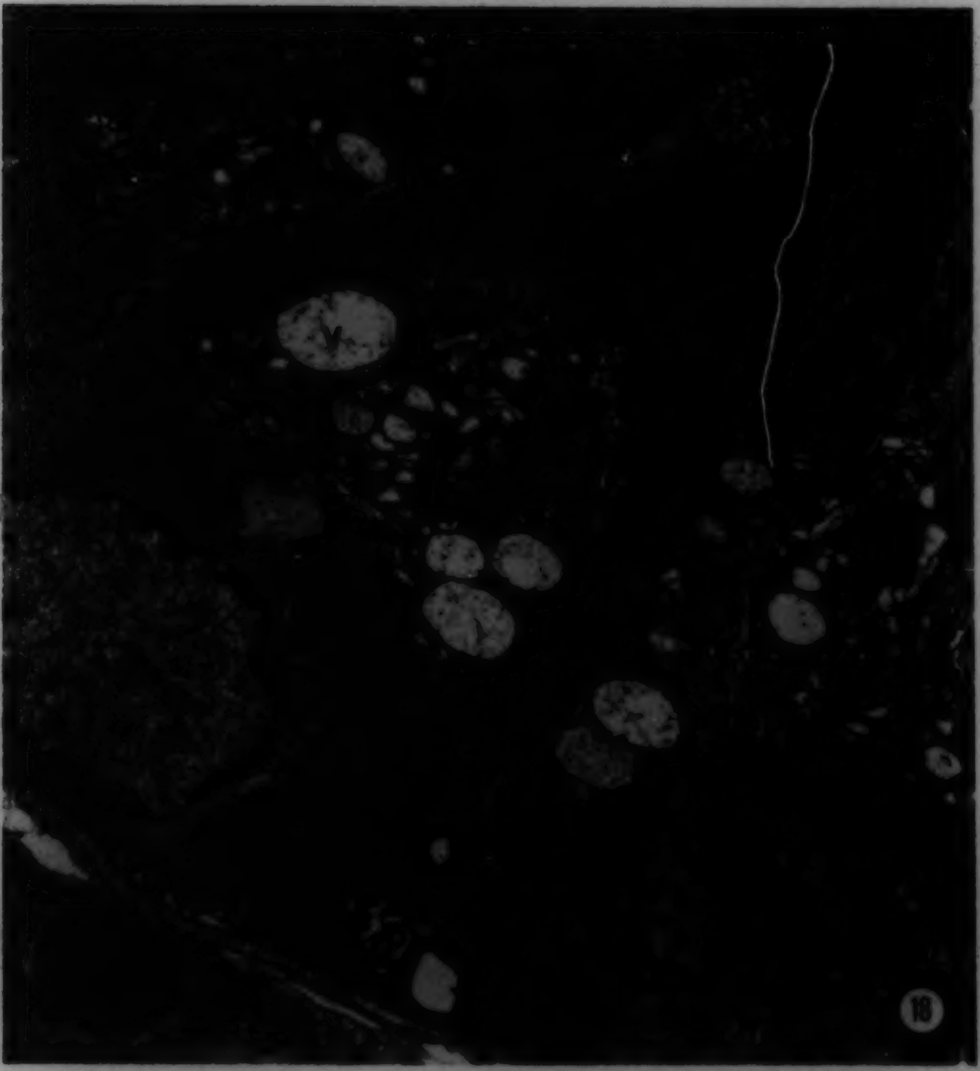
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Fig. IV-19. Ventral prostate-intact, short days: Note the changes in the height and width of the secretory epithelial cells, the reduction in the amount of the infranuclear cytoplasm, and paucity of the rough endoplasmic reticulum (RER), particularly in the perinuclear area, relative to those seen in long days. The Golgi complex (G) consists of groups of short stacked cisternae. SG, secretory granules; V, condensing vacuoles. Bar equals 2 μm.

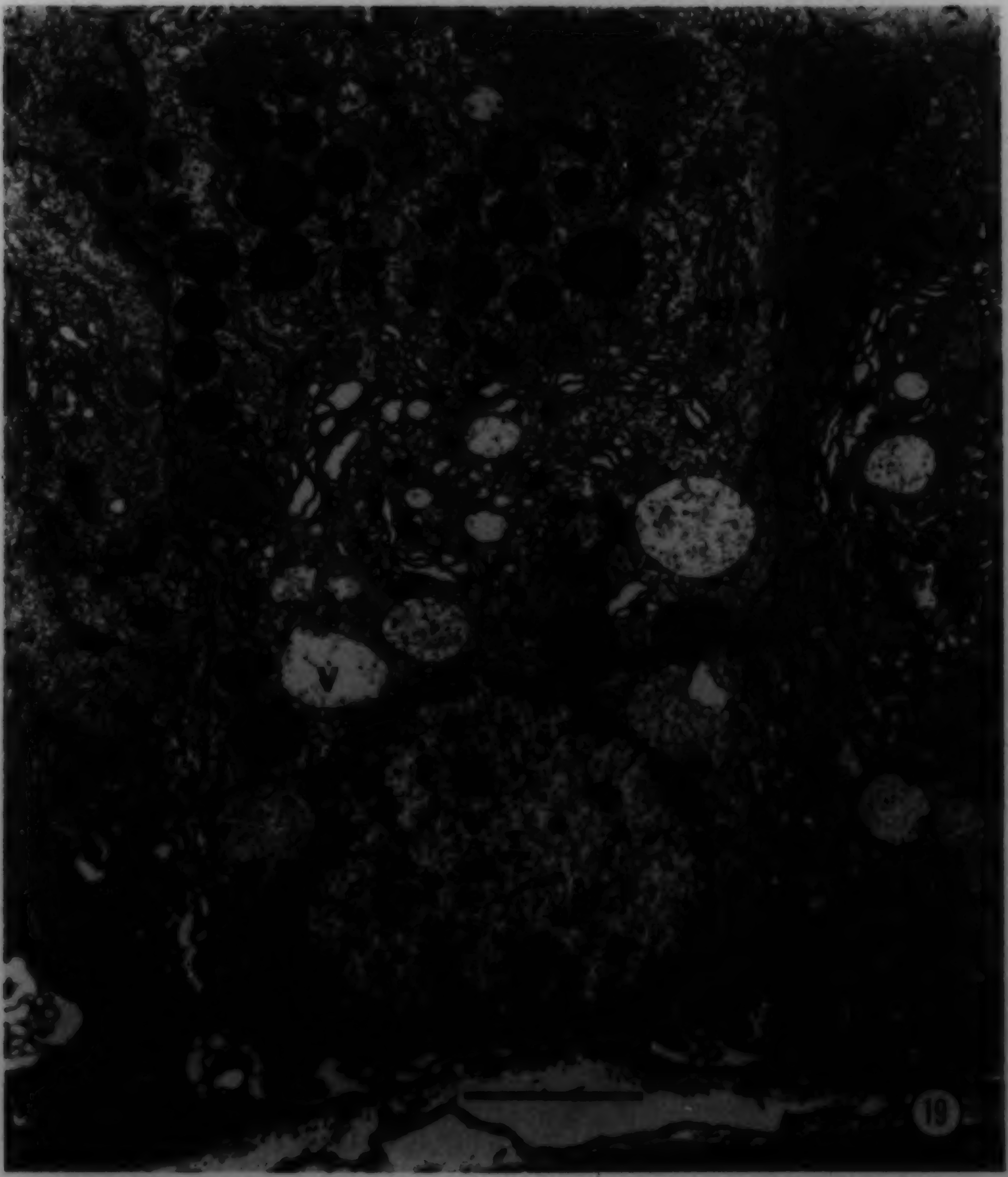
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Fig. IV-20. Ventral prostate-intact, short days: Supranuclear and apical portions of secretory epithelial cell. Note the reduction in the profiles of rough endoplasmic reticulum (RER). The Golgi complex (G) is reduced to short collapsed stacks of cisternae. A number of secretory granules (SG) are present in the apical cytoplasm. Note the marked reduction in the number of the large condensing vacuoles that are normally associated with Golgi complex of intact animals kept in long days. Bar equals 1.5 μm.

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Fig. IV-21. Ventral prostate-intact, short days: The cytoplasm of the secretory cells appears less electron-dense than that of animals kept in long days. Note the marked involution of the Golgi complex (G) and paucity of secretory granules (arrowheads). Note also the involution of the rough endoplasmic reticulum in the apical cytoplasm. Few vesicular profiles of this organelle are present in the apical cytoplasm (arrows). M, mitochondria; V, vacuole. Bar equals 1.5 μm.

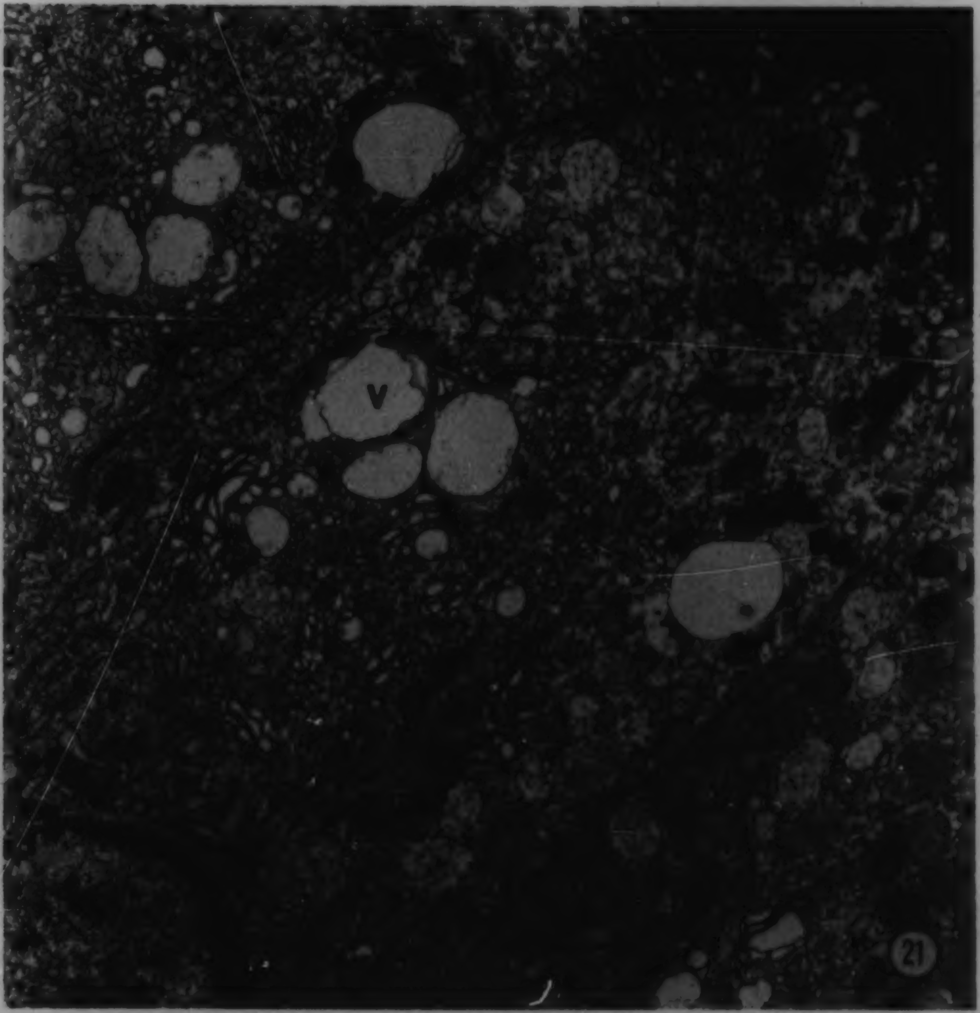
V
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Fig. IV-22. Ventral prostate-intact, short days: Note the dense bodies (DB) in the supranuclear area of the secretory cell. M, mitochondria. Bar equals 1.5 μm.

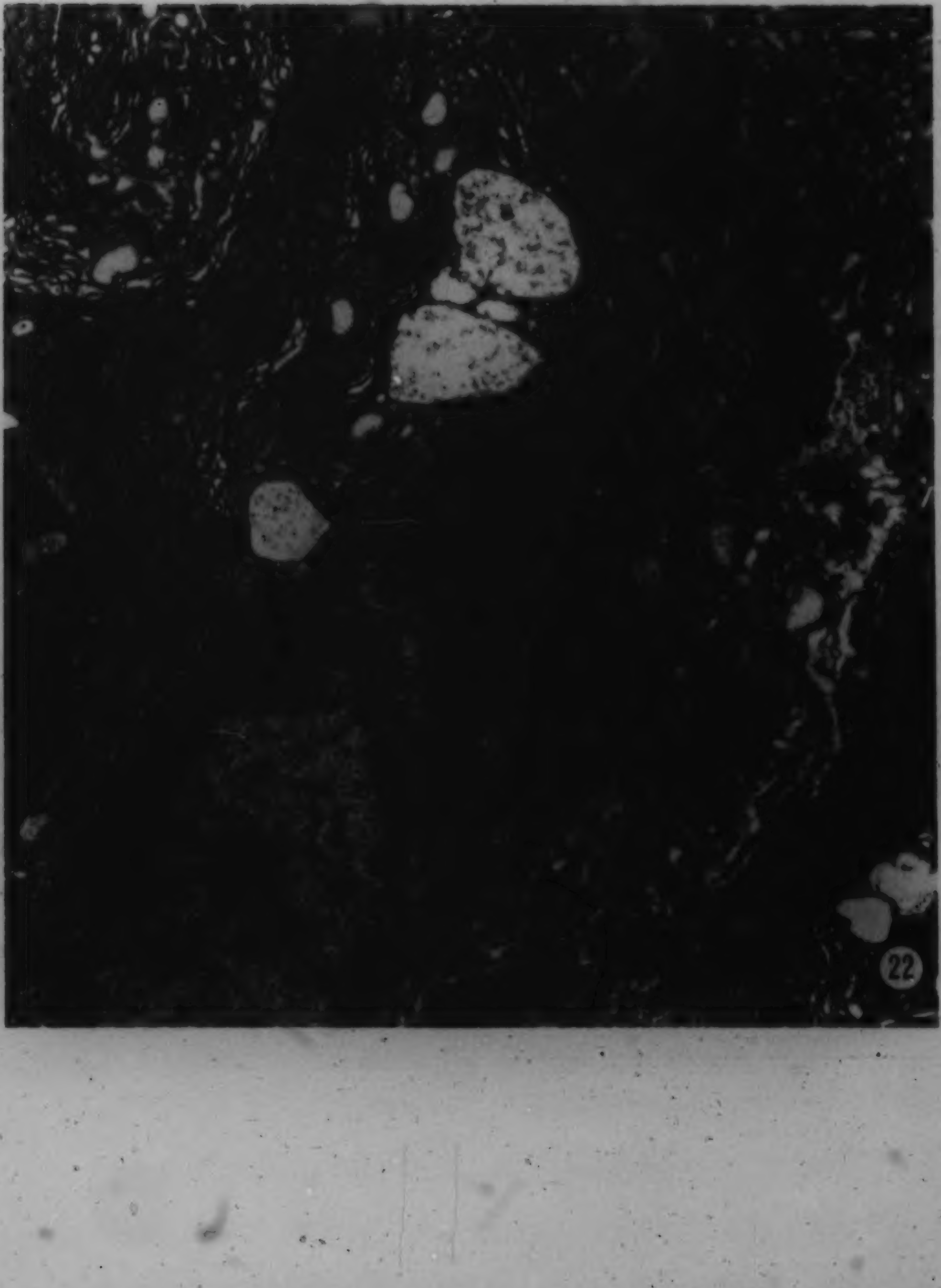

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Fig. IV-23. Ventral prostate-intact, short days: Basal portion of secretory epithelial cells. Note the large number of dense bodies (DB) with different sizes and configurations. Note also the large clumps of chromatin in the nuclei (open arrows). Bar equals 1.5 μm.

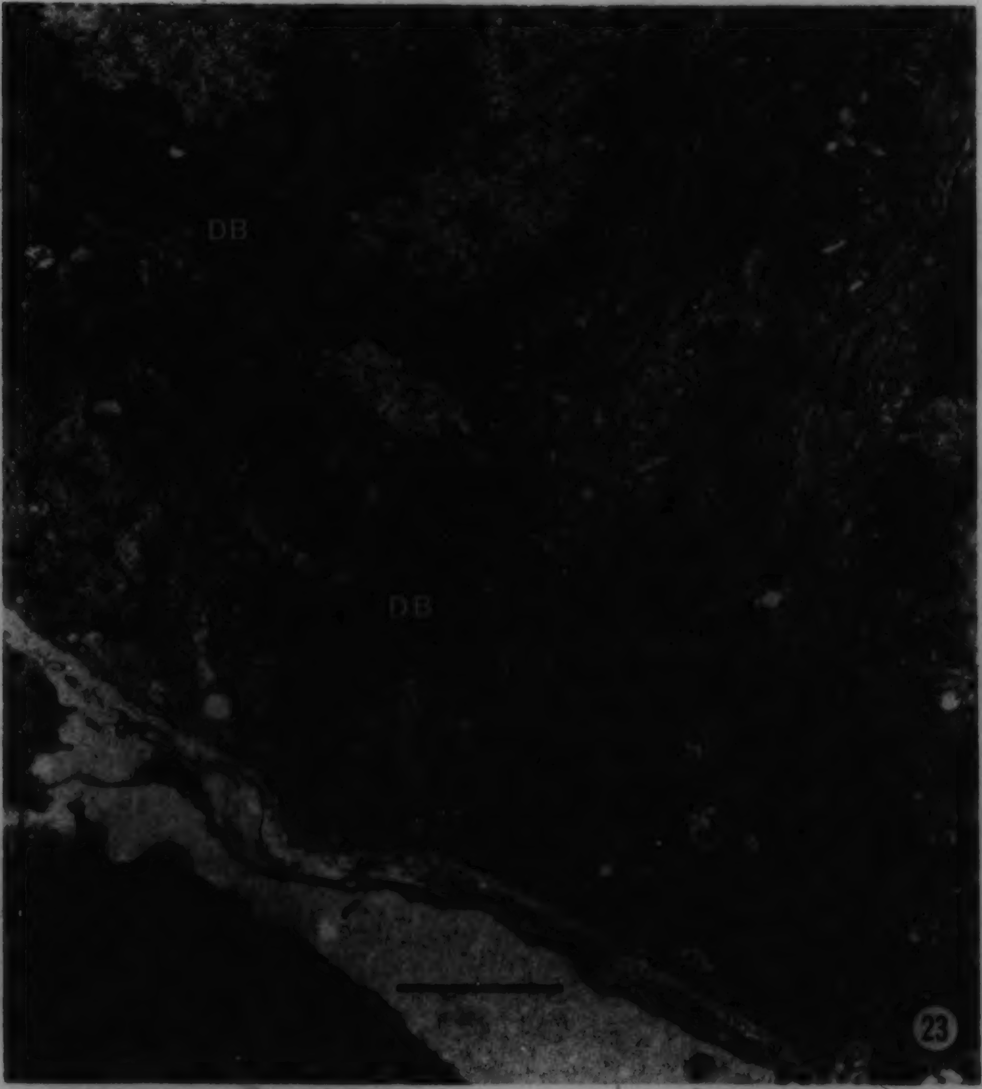

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Fig. IV-24. Ventral prostate-intact, short days: Note the dense body indicated by the arrowhead contains numerous small vesicle and vacuoles. The section also illustrate signs of cytoplasmic dissolution in the central portion of the rough endoplasmic reticulum-whorls (*), forming a large pool of amorphous material. DB, dense body. Bar equals 1.5 μm.

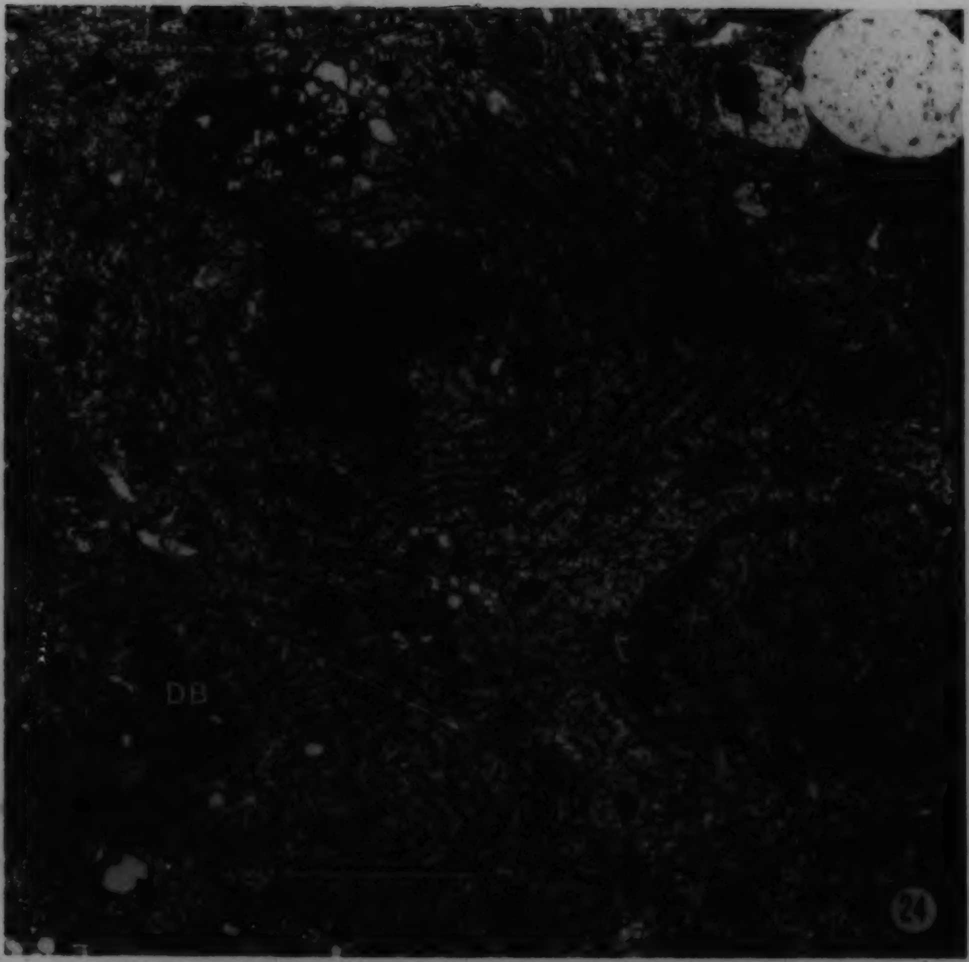
DB
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Fig. IV-25. Ventral prostate-pinealectomy, short days: Note the extensive development of the rough endoplasmic reticulum (RER), and the large Golgi area (G). The Golgi complex consists of large dilated membranous sacs, and several condensing vacuoles and vesicles. Bar equals 4 μm.

25

Fig. IV-26. Ventral prostate-pinealectomy, short days: Section through the nuclear and supranuclear regions of secretory epithelial cells. Note the extensive development of the rough endoplasmic reticulum and the concentric lamellated profiles in the supranuclear area. Bar equals 1 μm.

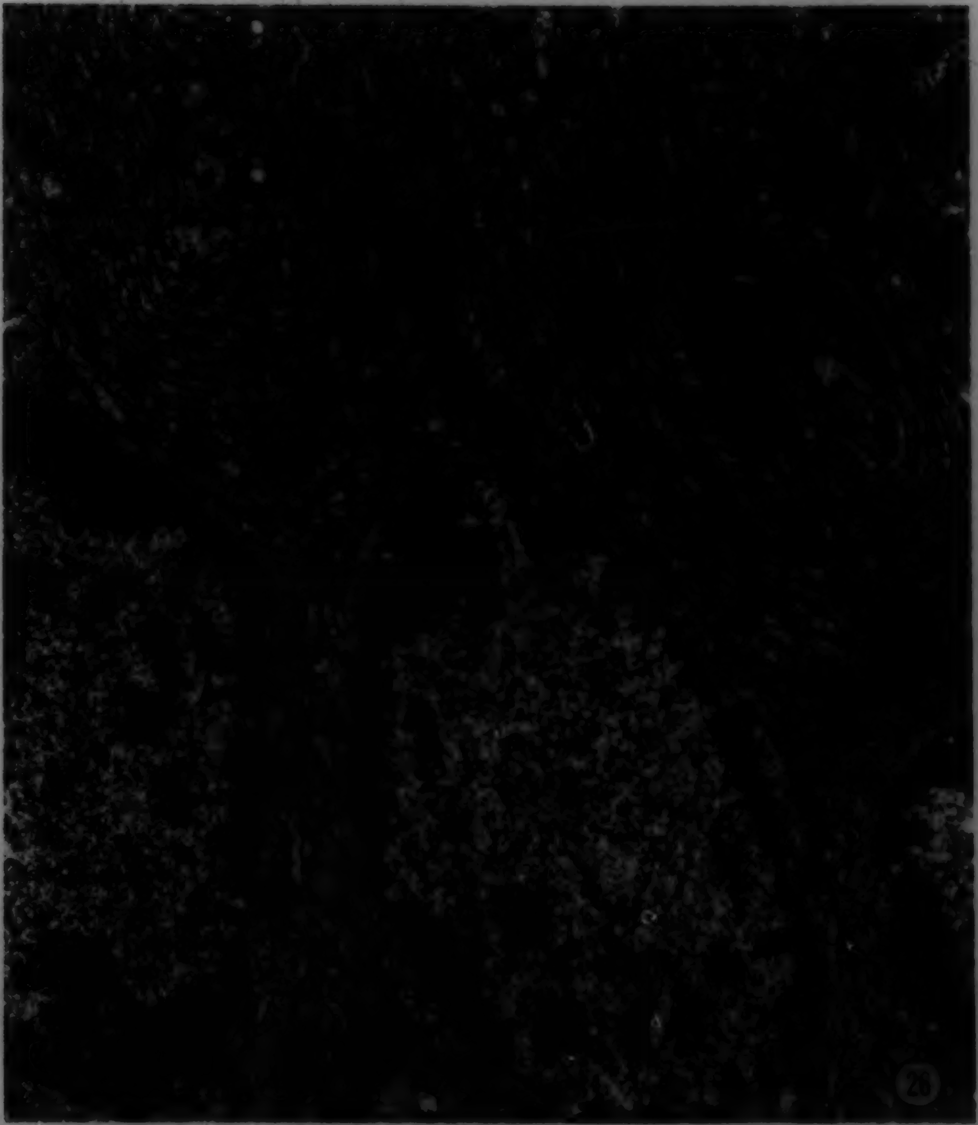

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Fig. IV-27. Ventral prostate-pinealectomy, short days: Detail of the infranuclear regions of two adjacent secretory cells. Note the presence of vesicular (arrowhead) and lamellar profiles of rough endoplasmic reticulum (RER). Bar equals 1 μm.

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Fig. IV-28. Ventral prostate-pinealectomy, short days: Detail of apical portion of secretory epithelial cells. Note cisternal arrangement of the rough endoplasmic reticulum (RER), and its orientation to the lateral plasma membrane. Vesicular profiles of RER are visible at arrowheads. Several secretory granules (SG) are seen in this field. Bar equals 1 μm.

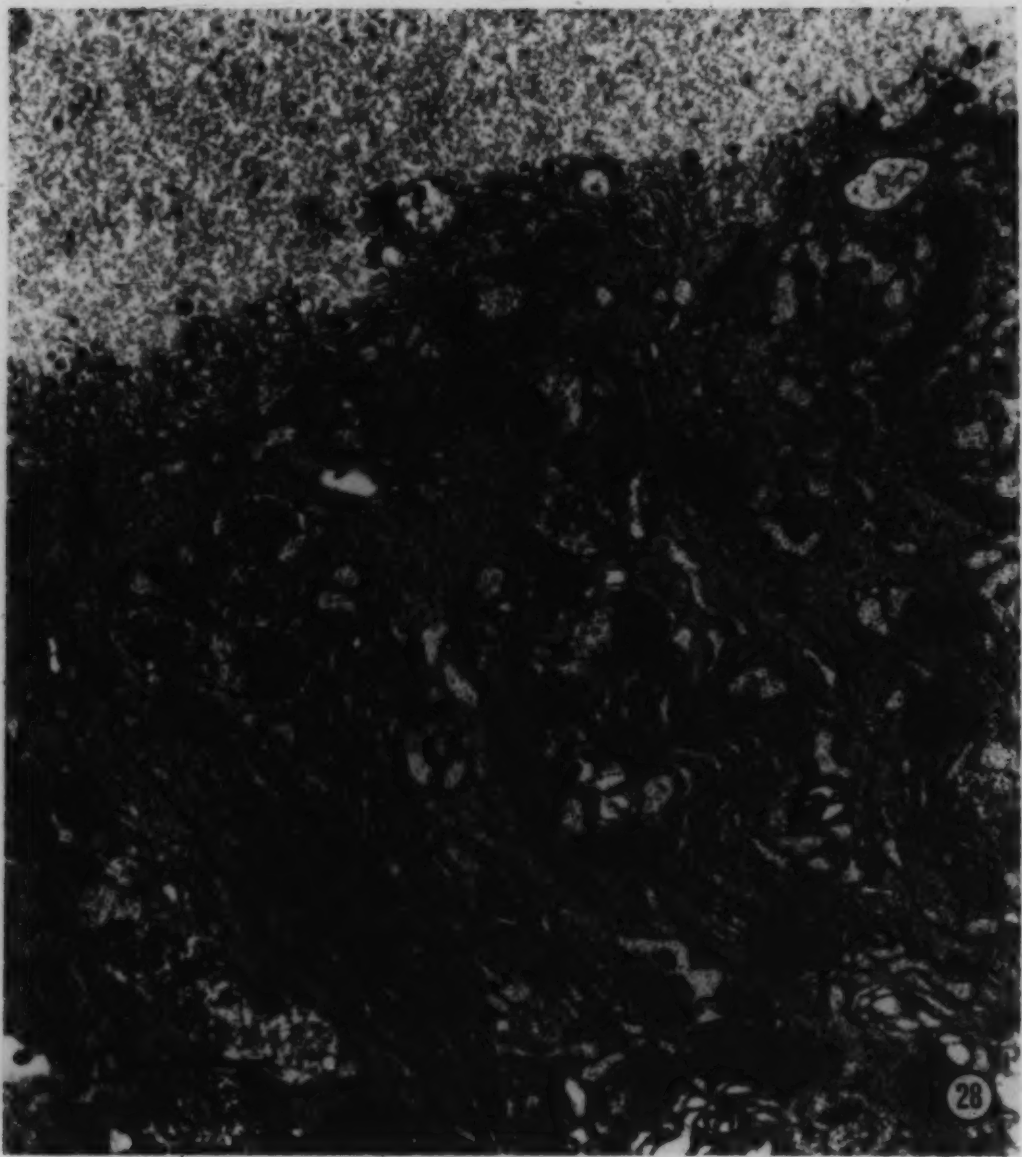

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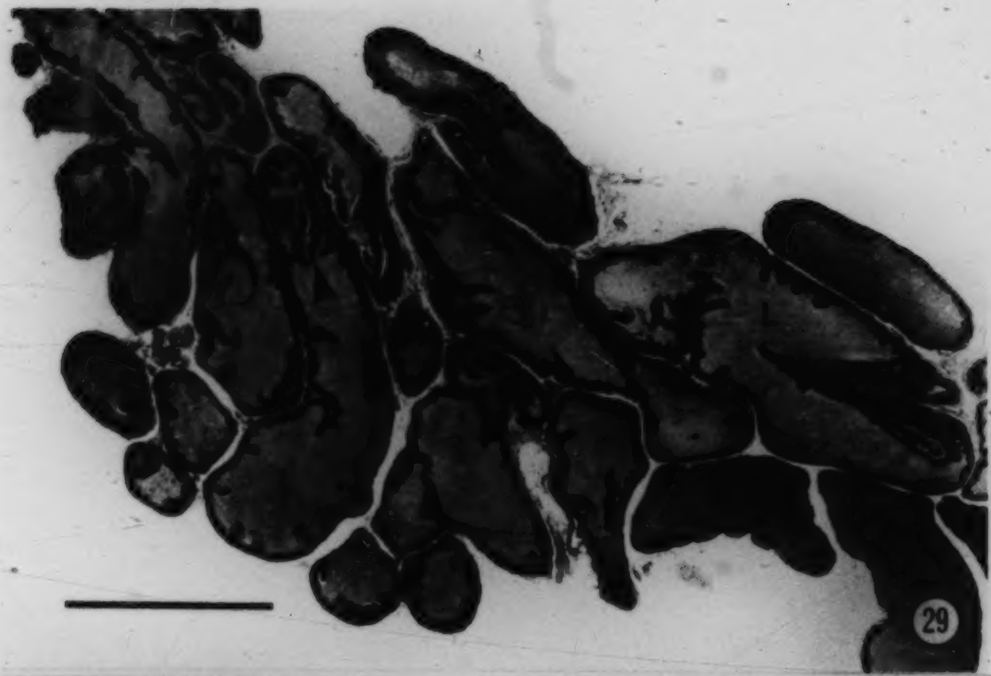
Fig. IV-29. Dorsal prostate-intact, long days: Survey light micrograph of the dorsal prostatic acini. Note the differences in the degree of the mucosal foldings among different acini (arrowheads). The acinar lumen (L) is filled with secretory products. Bar equals 1 mm.

Fig. IV-30. Dorsal prostate-intact, long days: Light micrograph of a portion of acinar wall with folded mucosa. The epithelium (E) is represented by tall columnar secretory cells, and occasional basal cells (BC). The secretory cells contain basally located nuclei and pools of amorphous material distributed throughout the cytoplasm (arrows). L, acinar lumen; LP, lamina propria; SM, smooth muscle fibers of the muscular stroma. Bar equals 20 μm.

Fig. IV-31. Dorsal prostate-intact, long days: Light micrograph of a portion of acinar wall with flattened mucosa. The epithelium (E) shows short columnar secretory cells and a basal cell (BC). Note the large pools of amorphous material in the basal cytoplasm of the secretory cells (arrows). L, acinar lumen; SM, smooth muscle fibers of the muscular stroma. Bar equals 20 μm.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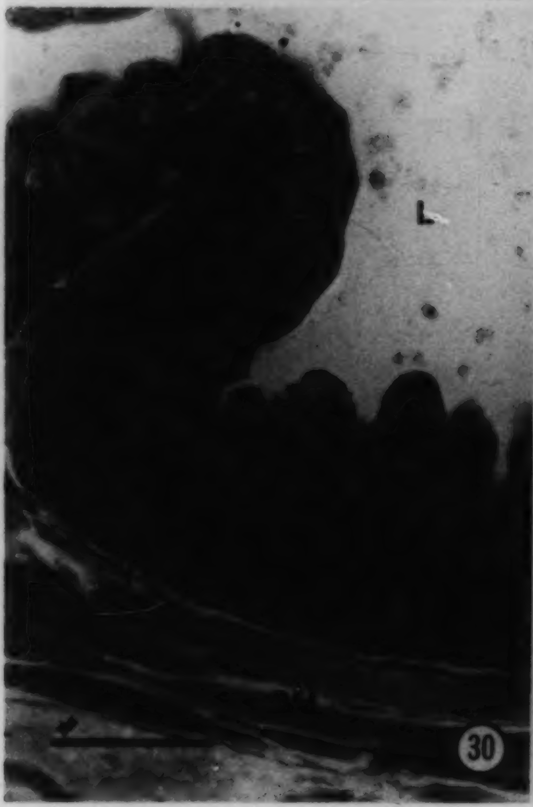
L
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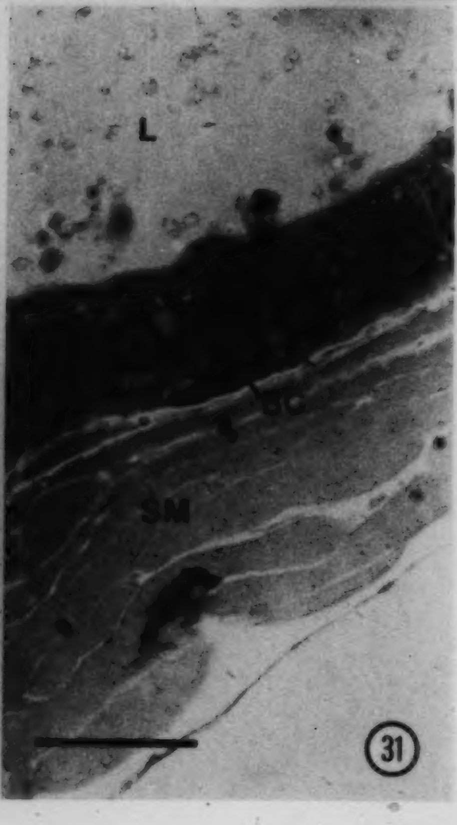
L
BC
SM
31

Fig. IV-32. Dorsal prostate-intact, long days: Survey electron micrograph of the short columnar secretory cells and part of the underlying lamina propria (LP) and smooth muscle fibers (SM) of the muscular stroma. The secretory cells show extremely dilated cisternae of rough endoplasmic reticulum (RER) which correspond to the pools of amorphous material seen in Fig. IV-31. The luminal surface shows microvilli and an apical bulge. Note the homogeneous cytoplasm in the distal portion of the bulge (*). G, Golgi complex; L, acinar lumen; Ly, lysosome. Bar equals 4 μm .

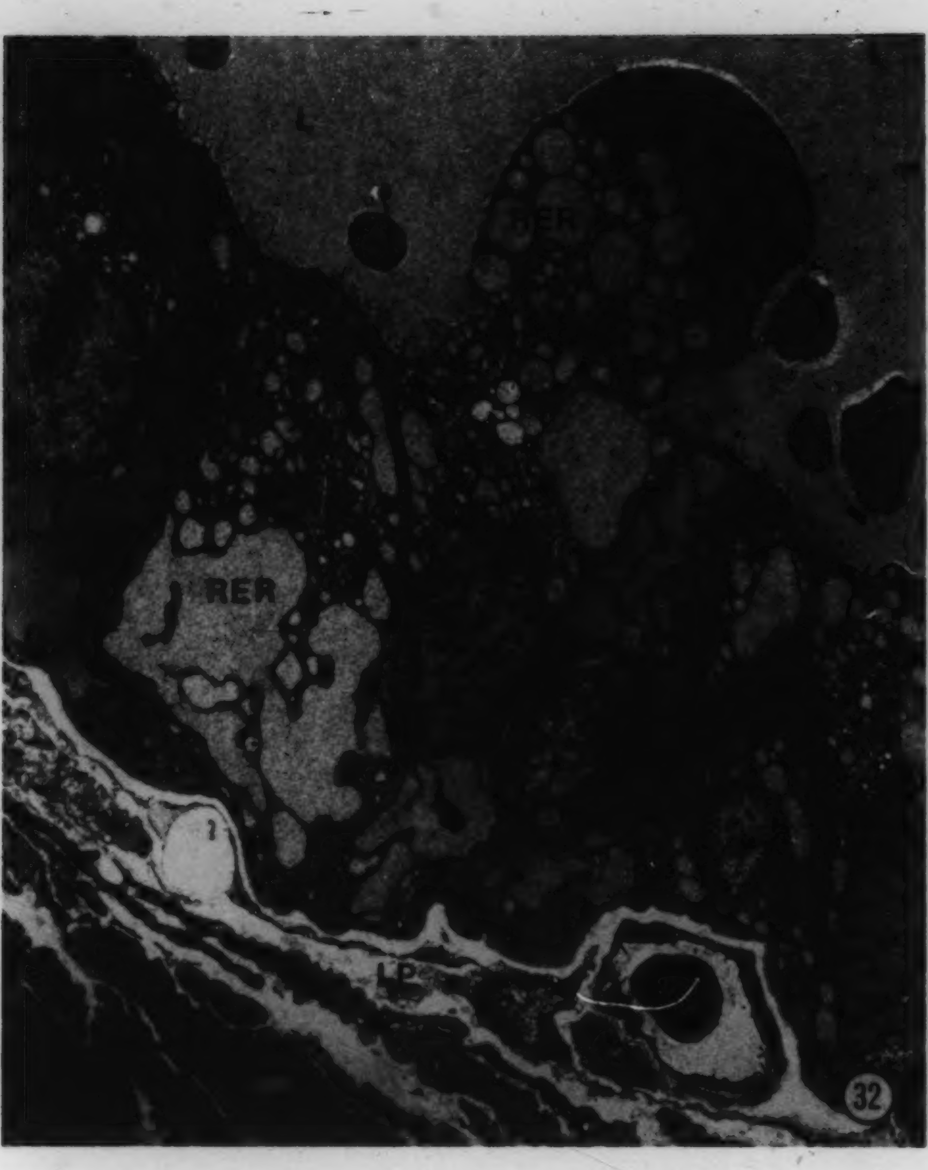

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Fig. IV-33. Dorsal prostate-intact, long days: Survey electron micrograph of tall columnar secretory cells. Note the basally placed nuclei, and the small dilated cisternae of rough endoplasmic reticulum (RER) which are dispersed in the cytoplasm. The Golgi complex (G) is small. An apical bulge containing amorphous material is visible at the luminal surface(*). L, acinar lumen; LP, lamina propria. Bar equals 5 μm.

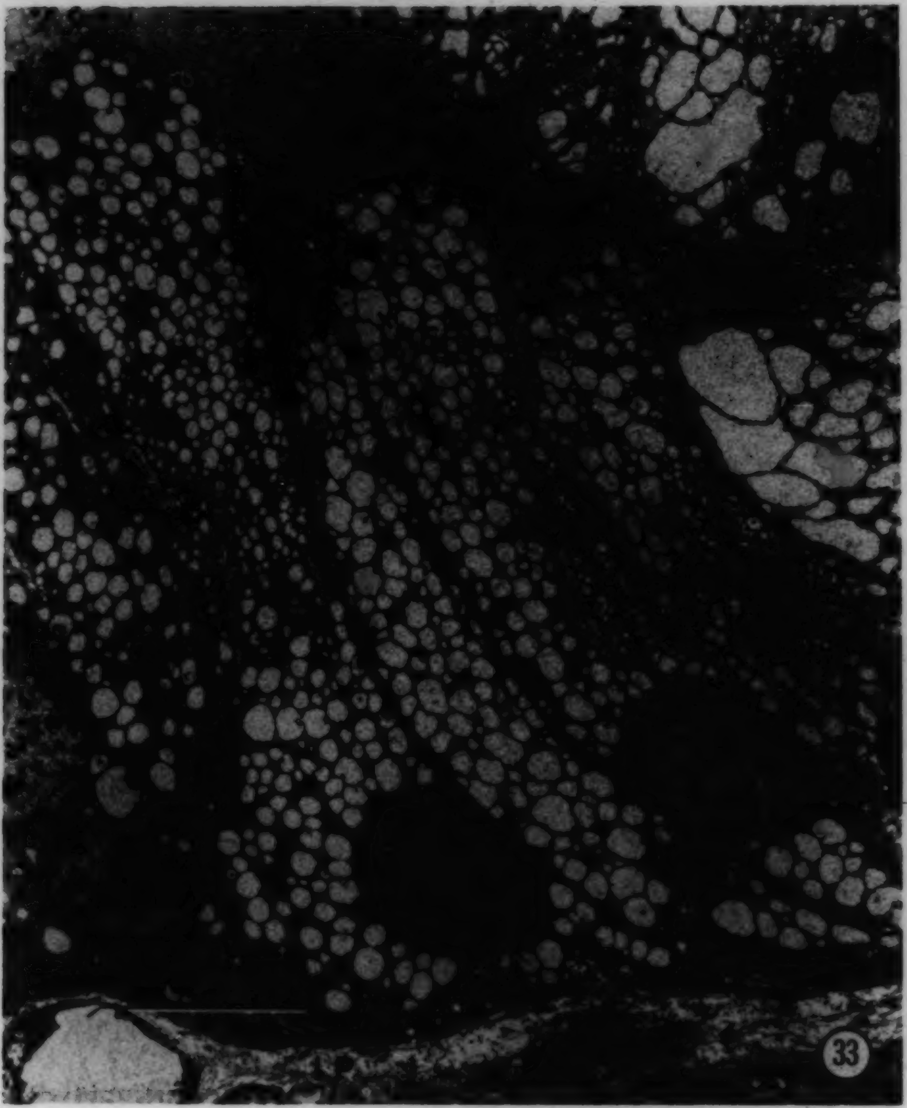
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Fig. IV-34. Dorsal prostate-intact, long days: Apical bulges containing organelles at the apical surface of secretory epithelial cells. The bulge on the right could be interpreted as an early form of apical bleb. The bulge on the left shows constriction at the base (arrowhead) which may represent a more advanced stage in bleb formation. Ly, lysosome. Bar equals 2 μm.

Fig. IV-35. Dorsal prostate-intact, long days: Apparent release of contents of a free bleb (arrow), into the acinar lumen (L). The bleb indicated by the open arrow shows a disintegrated limiting membrane. Bar equals 1 μm.

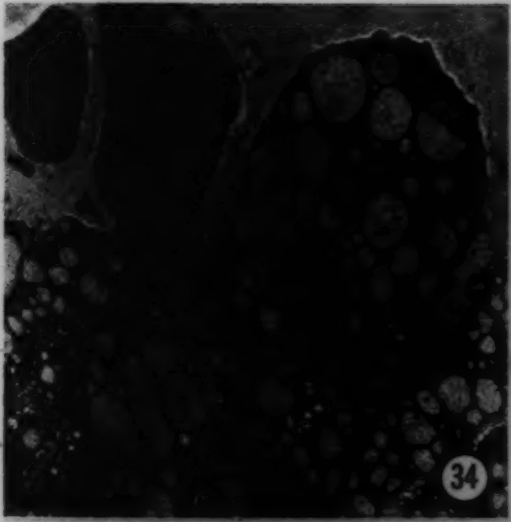
Fig. IV-36. Dorsal prostate-intact, long days: Various sizes and configurations of apical blebs containing granular material. Some of the blebs appear to be connected to the luminal surface by a narrow stalk (arrows). Bar equals 4 μm.

Fig. IV-37. Dorsal prostate-intact, long days: An apical bleb with irregular outlines, occupied by cytoplasmic matrix, small dilated cisternae of rough endoplasmic reticulum, and clusters of freely dispersed ribosomes. Bar equals 1 μm.

Fig. IV-38. Dorsal prostate-intact, long days: Contents of the acinar lumen close to the apical surface of secretory cells. Note the small apical blebs occupied by single dense bodies (open arrows). Bar equals 1 μm.

Fig. IV-39. Dorsal prostate-intact, long days: Contents of the acinar lumen close to the apical surface of secretory epithelial cells. Note the blebs contain single dense bodies (Open arrow), multivesicular bodies (arrowheads), or flocullent material (*). Bar equals 1 μm.

Fig. IV-40. Dorsal prostate-intact, long days: Portion of the luminal surface of a secretory epithelial cell. The microvilli show vesicular dilations at the distal end. A branched microvillus with vesicular tips is also evident (arrow). Bar equals 0.5 μm.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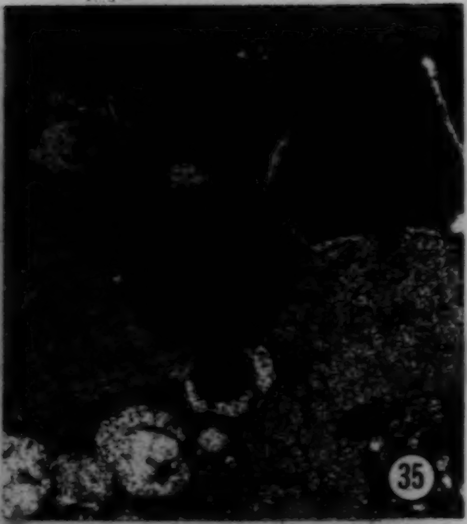
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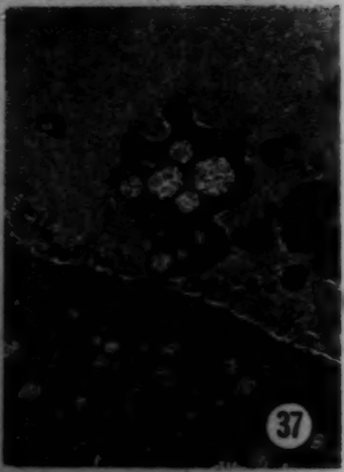
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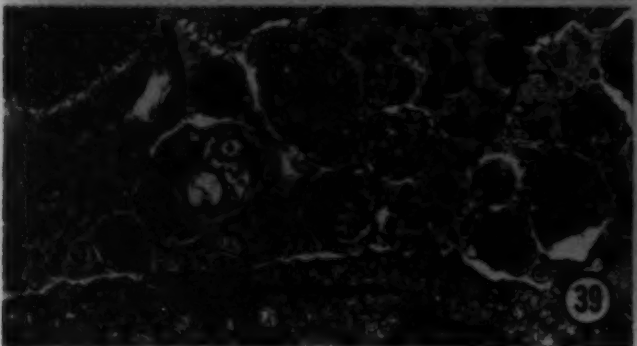
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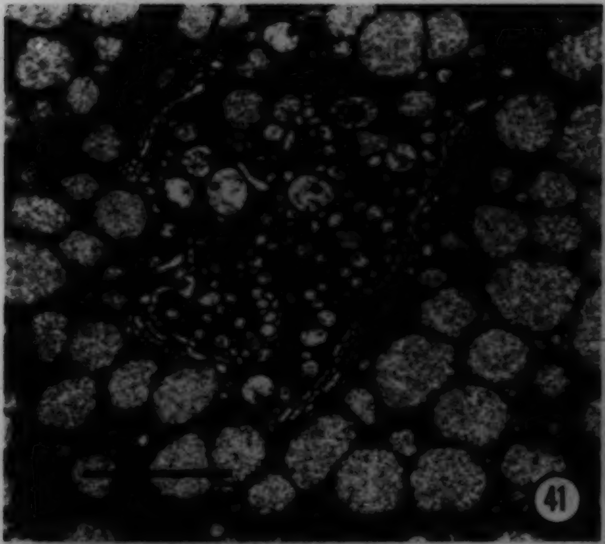
Fig. IV-41. Dorsal prostate-intact, long days: Portion of a secretory epithelial cell showing a moderately developed Golgi complex (G). Note the numerous stacked cisternae of the complex, and the secretory granules (arrowheads) which have dense cores surrounded by electron-translucent material. Bar equals 2 µm.

Fig. IV-42. Dorsal prostate-intact, long days: Portions of two secretory cells with poorly developed Golgi complexes (G), composed of few and short stacked cisternae. Secretory granules are absent. Bar equals 1 µm.

Fig. IV-43. Dorsal prostate-intact, long days: Apical portion of a secretory epithelial cell. Secretory granules are in close apposition to the apical plasmalemma (arrows). Bar equals 2 µm.

Fig. IV-44. Dorsal prostate-intact, long days: Apparent exocytosis of a secretory granule (open arrow). Bar equalls 0.5 µm.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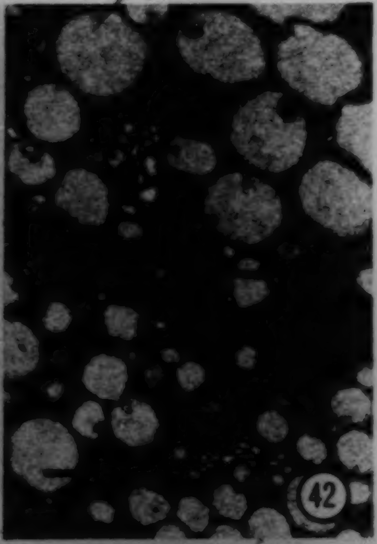
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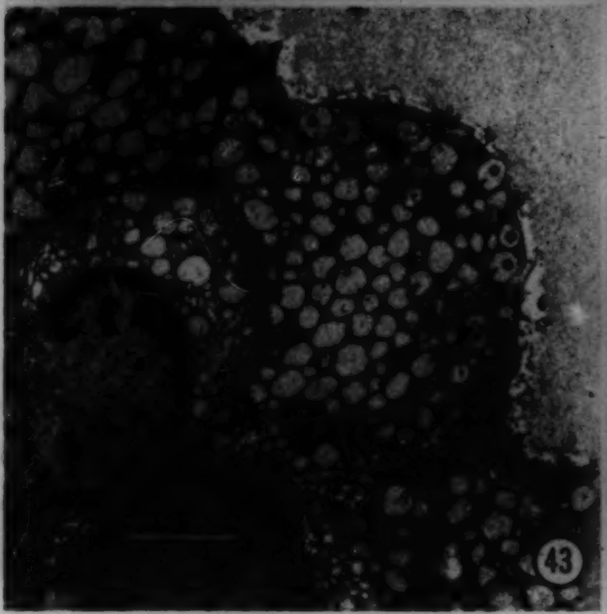
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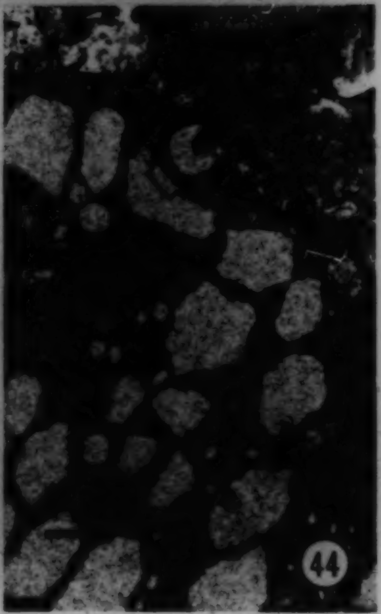
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Fig. IV-45. Dorsal prostate-intact, long days: Basal cell located between the basal portion of secretory cells and the basal lamina. The nucleus (N) is indented and shows an eccentrically located nucleolus (Nu). The cytoplasm contains small cisternae of rough endoplasmic reticulum, free ribosomes and mitochondria. Note the cytoplasmic processes of the basal cell (arrowheads) in relation to the basal portion of a secretory cell. BL, basal lamina. Bar equals 2 μm.

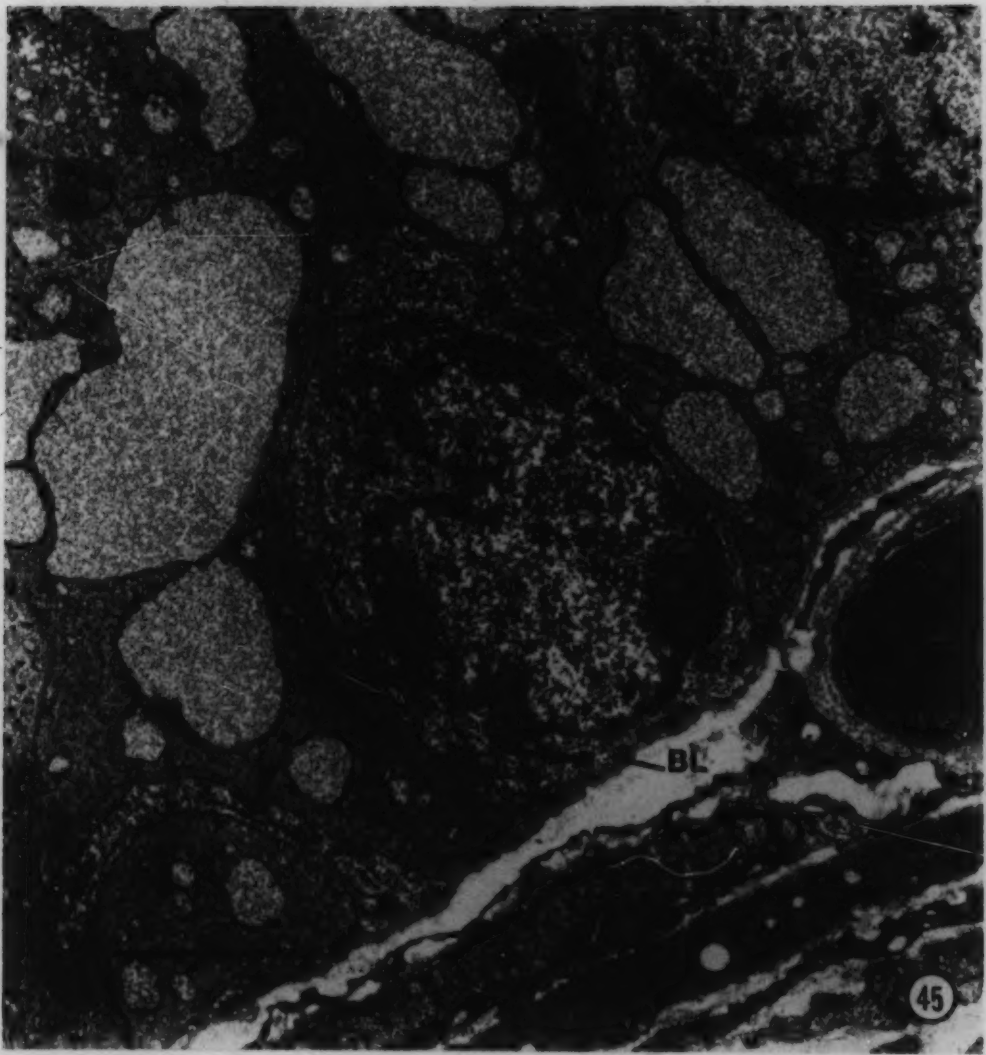
BL
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Fig. IV-46. Dorsal prostate-intact, short days: Note virtual absence of the large dilated cisternae of rough endoplasmic reticulum (RER) in the basal cytoplasm, compared with the short columnar cells of hamsters kept in long days. G, Golgi complex. Bar equals 3 μm.

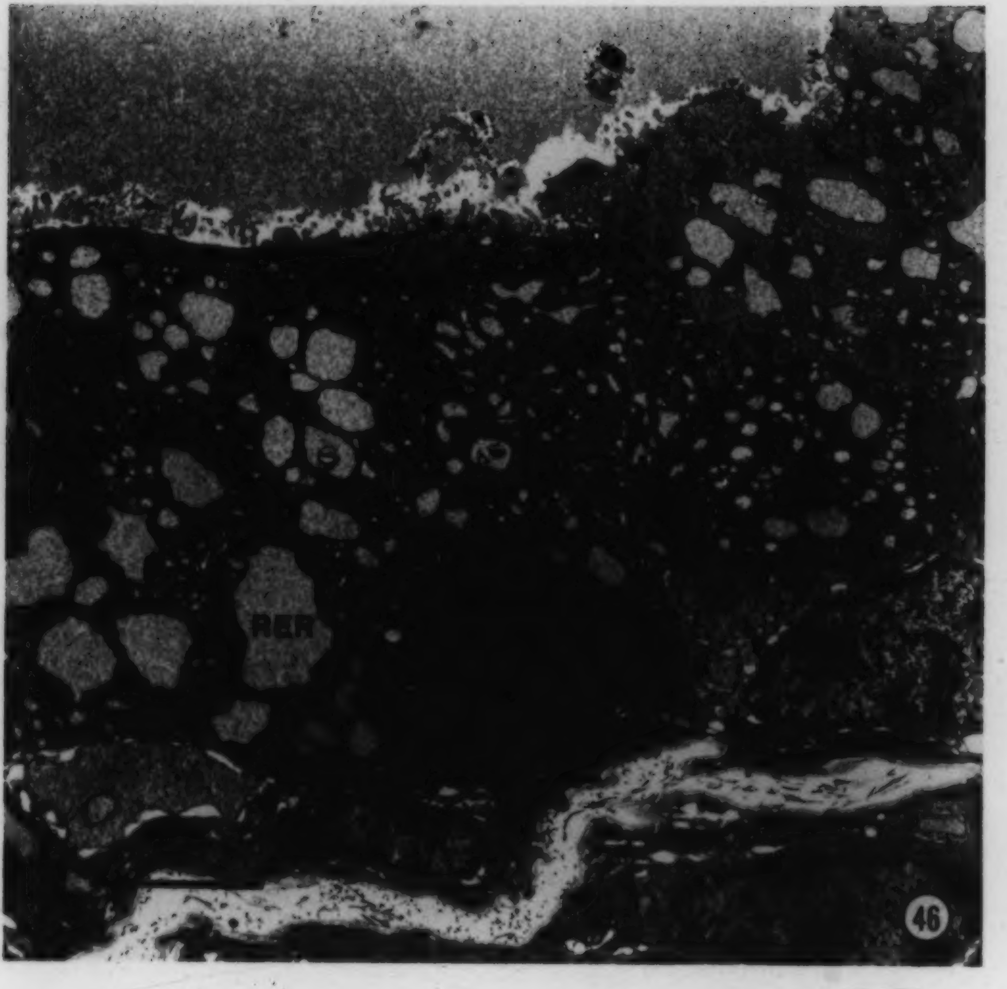
RER
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Fig. IV-47. Dorsal prostate-intact, short days: Secretory cells of the folded acini. Almost total destruction of the rough endoplasmic reticulum has occurred. Some of the nuclei (arrows) are crenated. Vesicles and membrane bound vacuoles (arrowheads) have accumulated in the apical cytoplasm. These may represent remnants of secretory vesicles or vacuolation due to autophagy. Bar equals 4 μm.

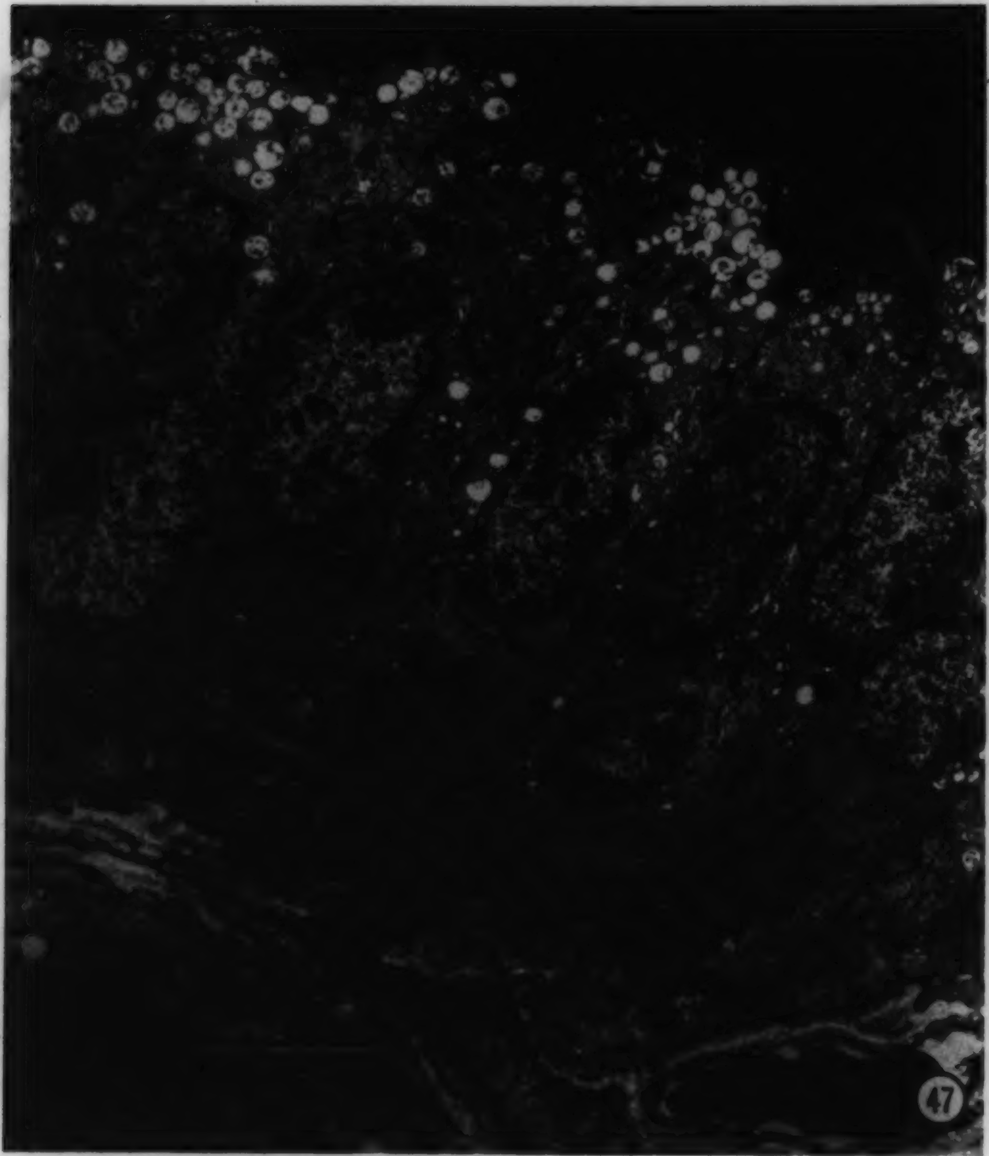

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Fig IV-48. Dorsal prostate-intact, short days: Secretory epithelial cells of the folded acini. The cells are almost deprived of rough endoplasmic reticulum, except for few and small dilated cisternae indicated at arrowheads. The microvilli at the luminal surface are barely detectable. Note the foldings of the basal plasma membrane (arrow). Bar equals 3 μm.

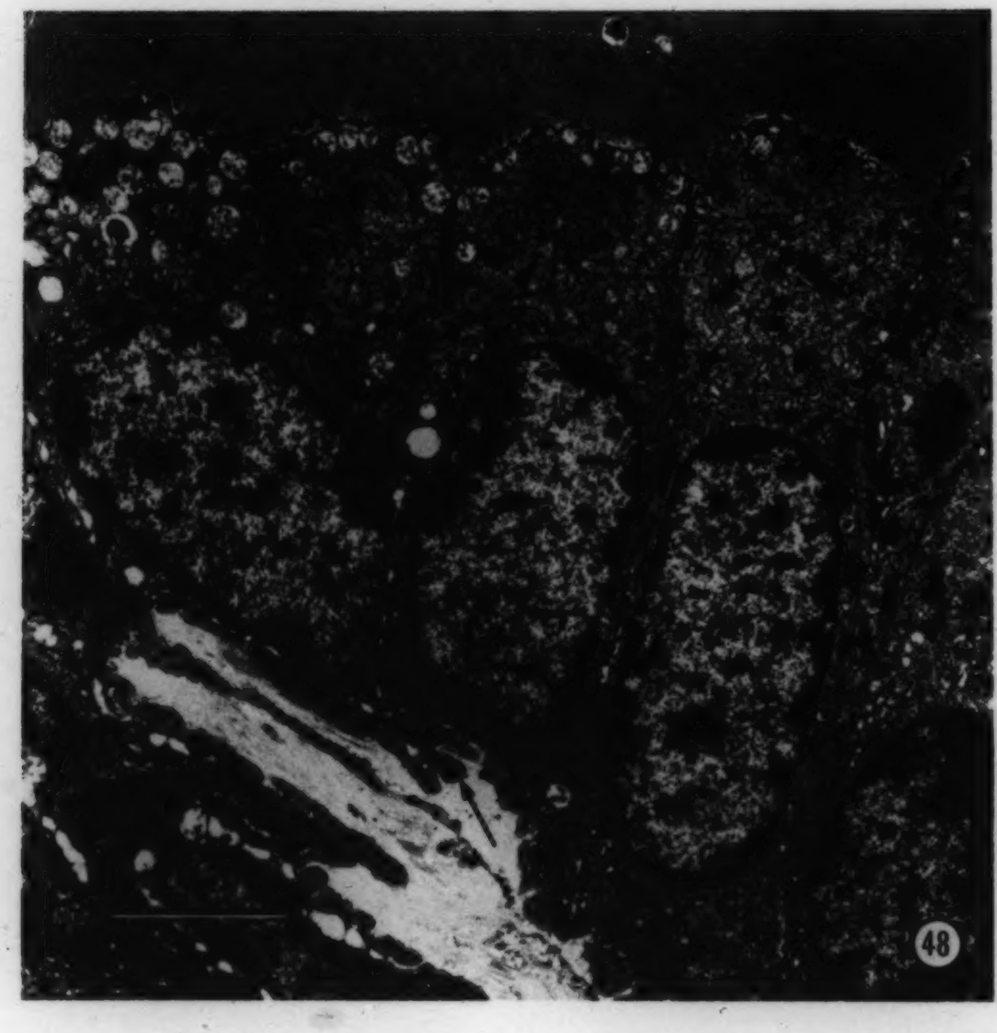

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Fig. IV-49. Dorsal prostate-intact, short days: Apical portion of a secretory epithelial cell. Note the partial degranulation of the endoplasmic reticulum membrane (open arrow), and the smooth luminal surface. L, acinar lumen. Bar equals 0.5 μm.

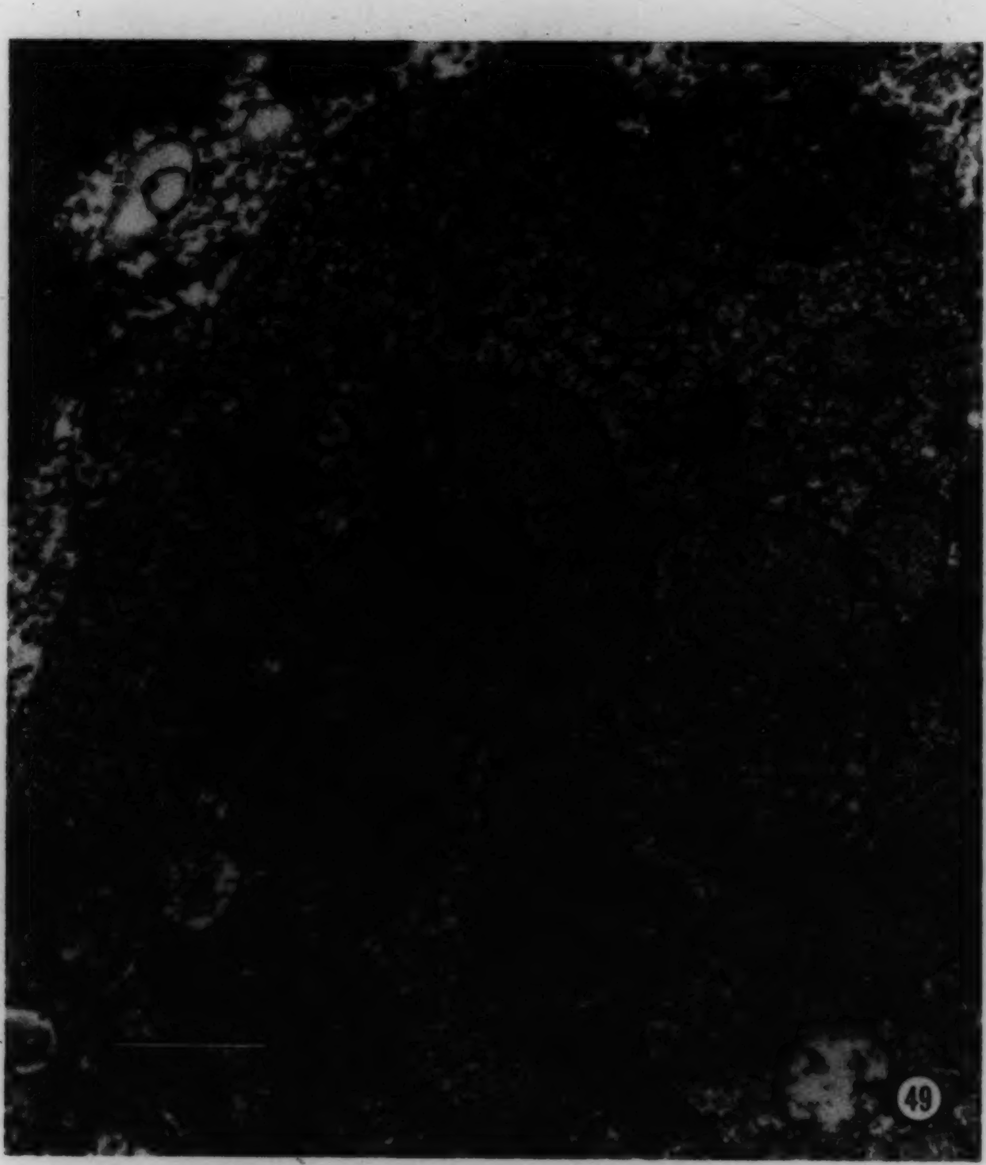
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Fig. IV-50. Dorsal prostate-intact, short days: Supranuclear and apical portion of a secretory epithelial cell. Note virtual absence of microvilli at the luminal surface, and total destruction of the Golgi complex has occurred. Only few and small dilated cisternae of rough endoplasmic reticulum are visible (arrowheads). Clusters of free ribosomes are dispersed throughout the cytoplasm. L, acinar lumen. Bar equals 1 μm.

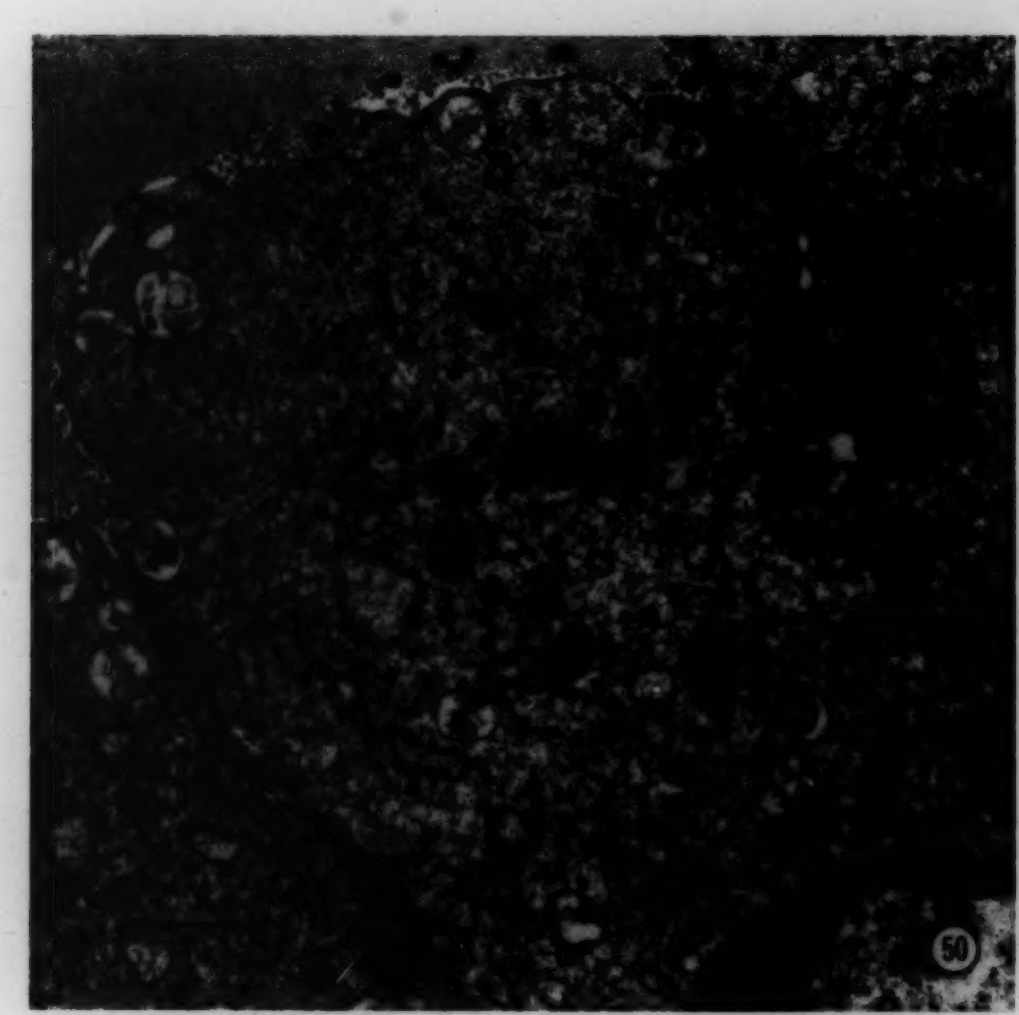

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Fig. IV-51. Dorsal prostate-intact, short days: Basal, nuclear, and supranuclear regions of the tall columnar secretory cells. Note the Golgi complex (G), and the collapsed fragments of rough endoplasmic reticulum (arrowheads). An increase in the number of lysosomes (open arrows) is evident, as compared with the secretory cells of hamsters maintained in long days. Bar equals 2 µm.

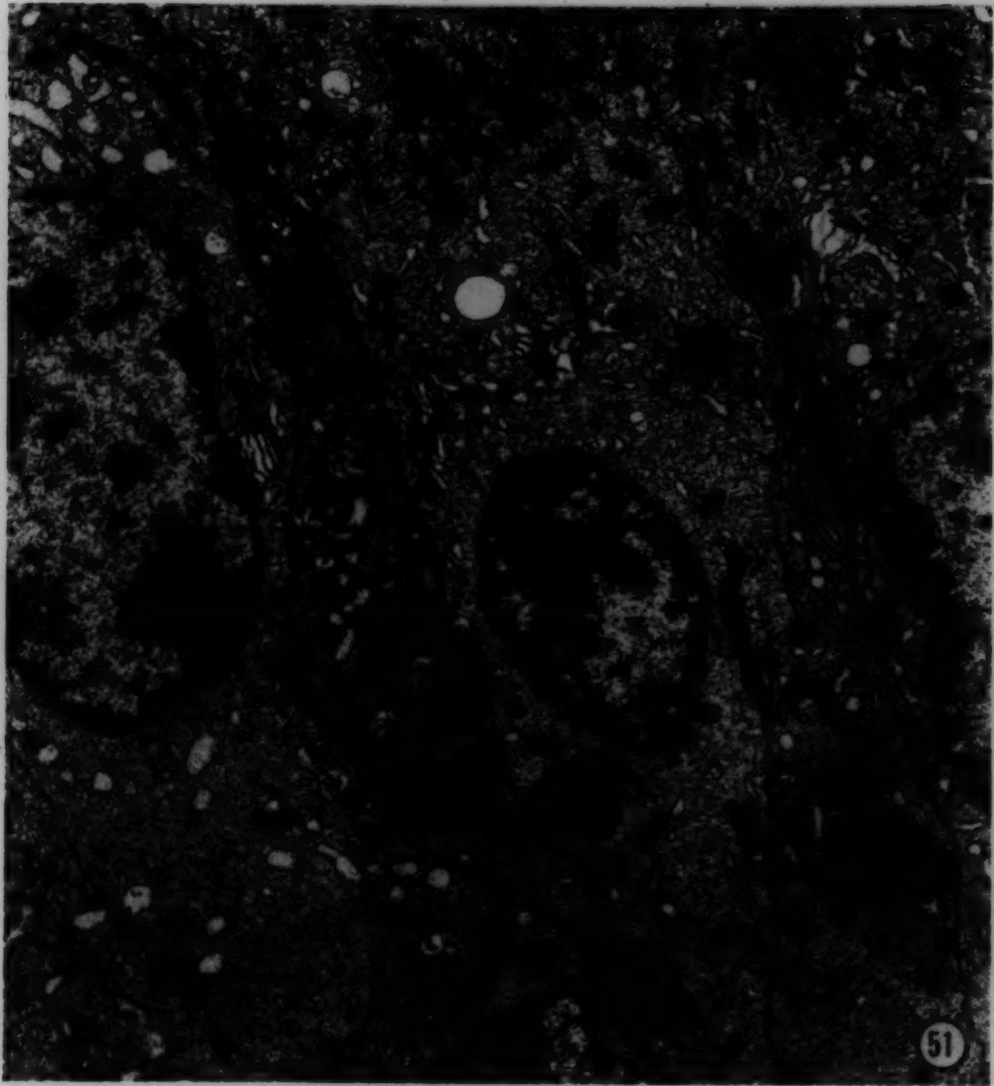

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Fig. IV-52. Dorsal prostate-intact, short days: Note the large autophagic vacuole located in the supranuclear portion of the secretory cell. It contains dense bodies and digested material. Bar equals 2 μm.

Fig. IV-53. Dorsal prostate-intact, short days: Note the dense body containing membranous formations. Bar equals 0.2 μm.

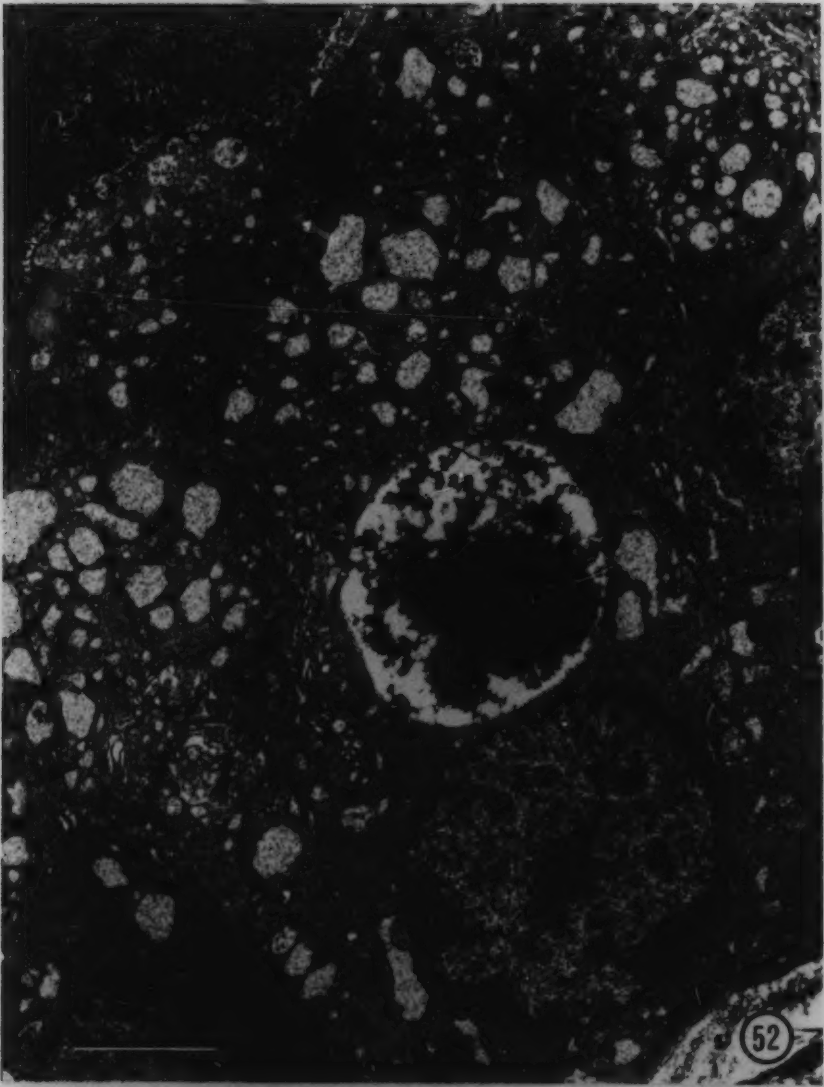
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Fig. IV-54. Dorsal prostate-intact short days: Basal surface membrane and basal lamina. The foldings of the cell base are obvious, and the basal lamina (arrowheads) follows the contours of the cell membrane. Bar equals 1 μm.

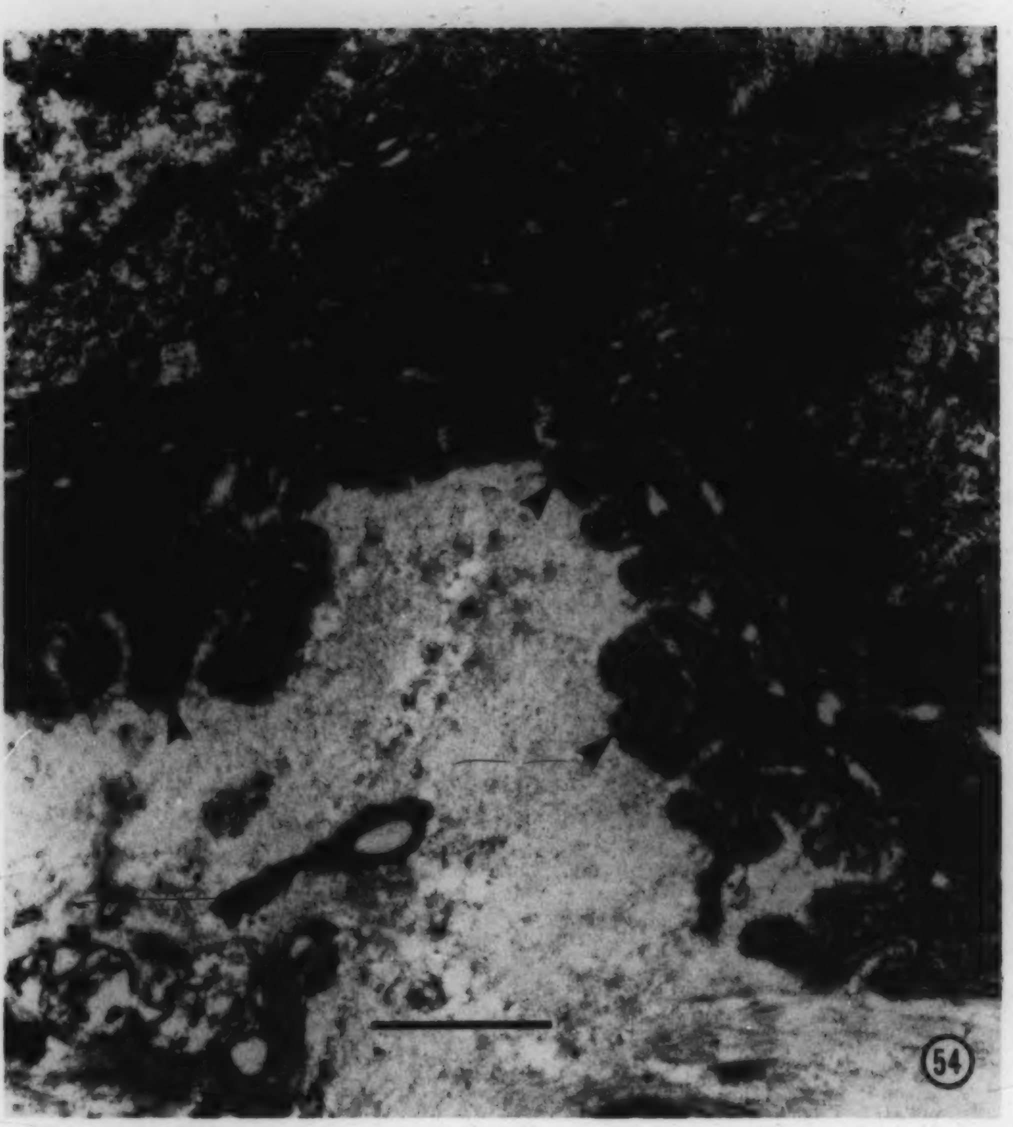
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Fig. IV-55. Dorsal prostate-pinealectomy, short days: Short columnar secretory cells of the distended acini. The cells are characterized by a highly dilated cisternae of rough endoplasmic reticulm (RER), a moderately developed Golgi complex (G), and the presence of microvilli, apical bulges and blebs at the luminal surface. L, acinar lumen. Bar equal 4 μm.

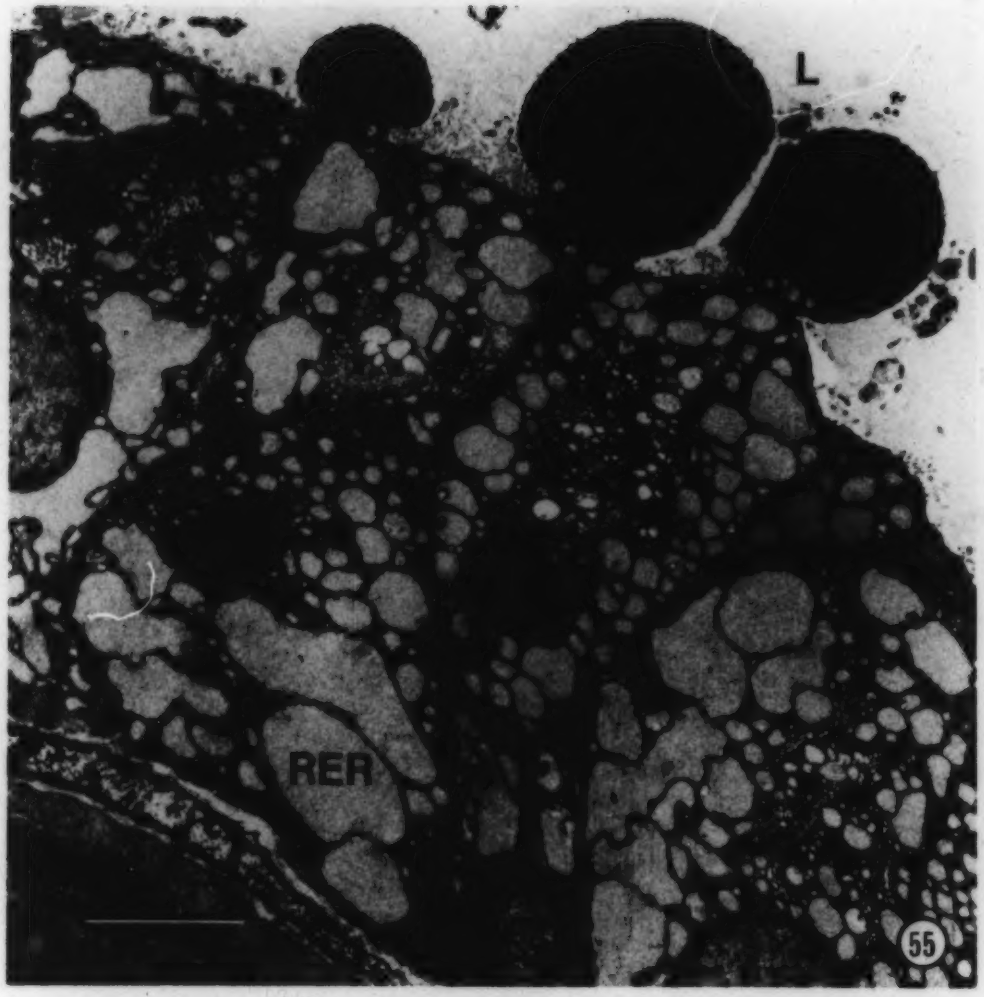

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Fig. IV-56. Dorsal prostate-pinealectomy, short days: The luminal surface shows numerous microvilli with vesicular tips. The cytoplasm of the apical bulge contains small dilated cisternae of rough endoplasmic reticulum and clusters of free ribosomes. The apical blebs (*) contain amorphous material. Free blebs are also seen in the acinar lumen (L). Bar equals 1.5 μm.

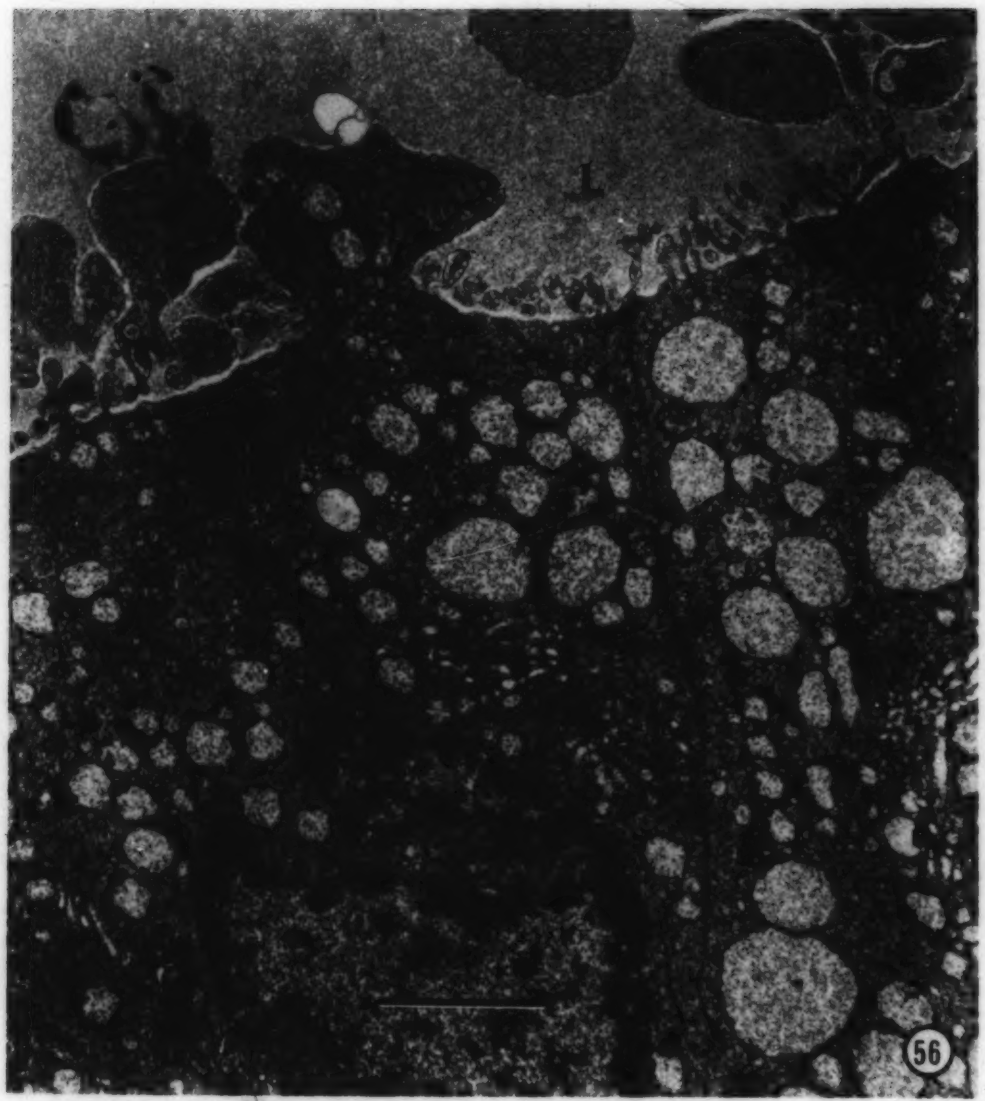

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Fig. IV-57. Dorsal prostate-pinealectomy, short days: Short columnar secretory cells. The dilated cisternae of rough endoplasmic reticulum (RER) occupy most of the cytoplasm except the Golgi area (G). The Golgi complex is well developed. The number of the secretory granules (arrowheads) in the Golgi and apical areas of the cells apparently have increased, as compared with those observed in animals maintained in long days. L., acinar lumen. Bar equals 4 μm.

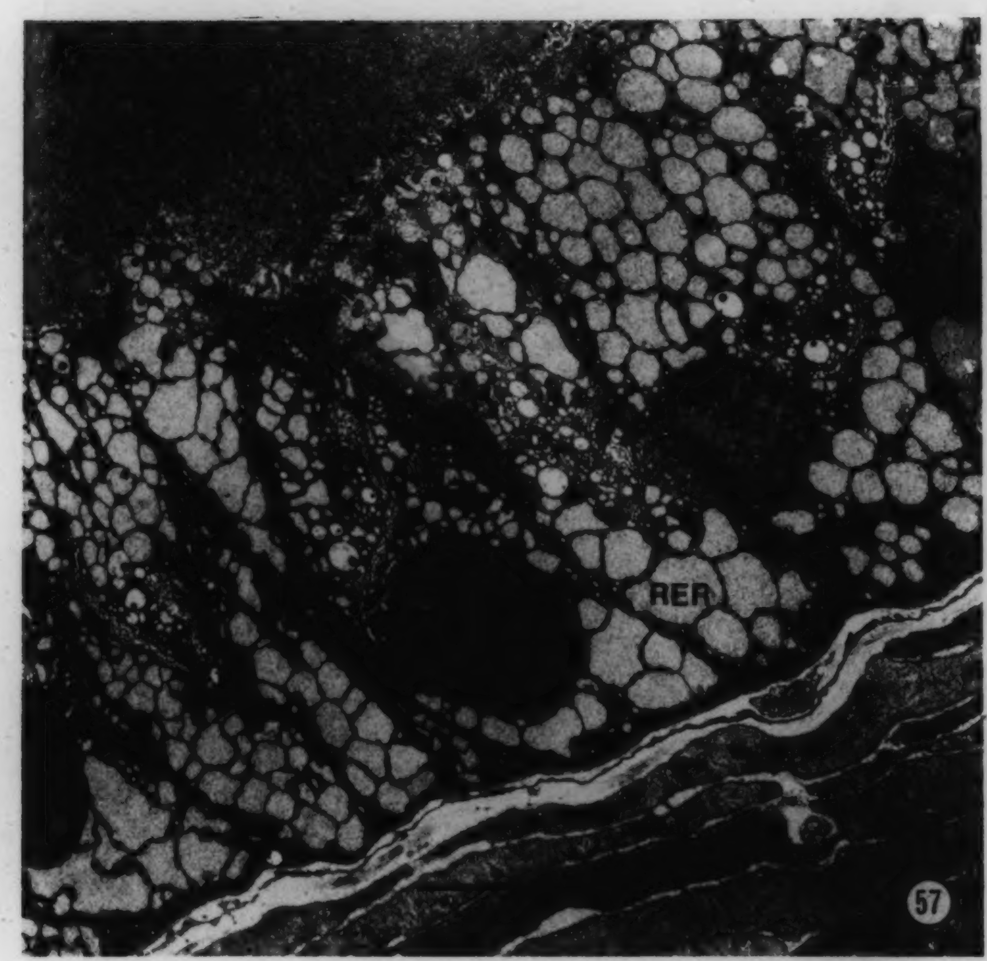
RER
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Fig. IV-58. Dorsal prostate-pinealectomy, short days: Section through the supranuclear and apical portions of the tall columnar secretory cells. Note the well developed Golgi complex (G), and the large number of secretory granules (arrowhead) in the apical cytoplasm. Some of these are in close apposition to the luminal plasma membrane. Bar equals 2 μm.

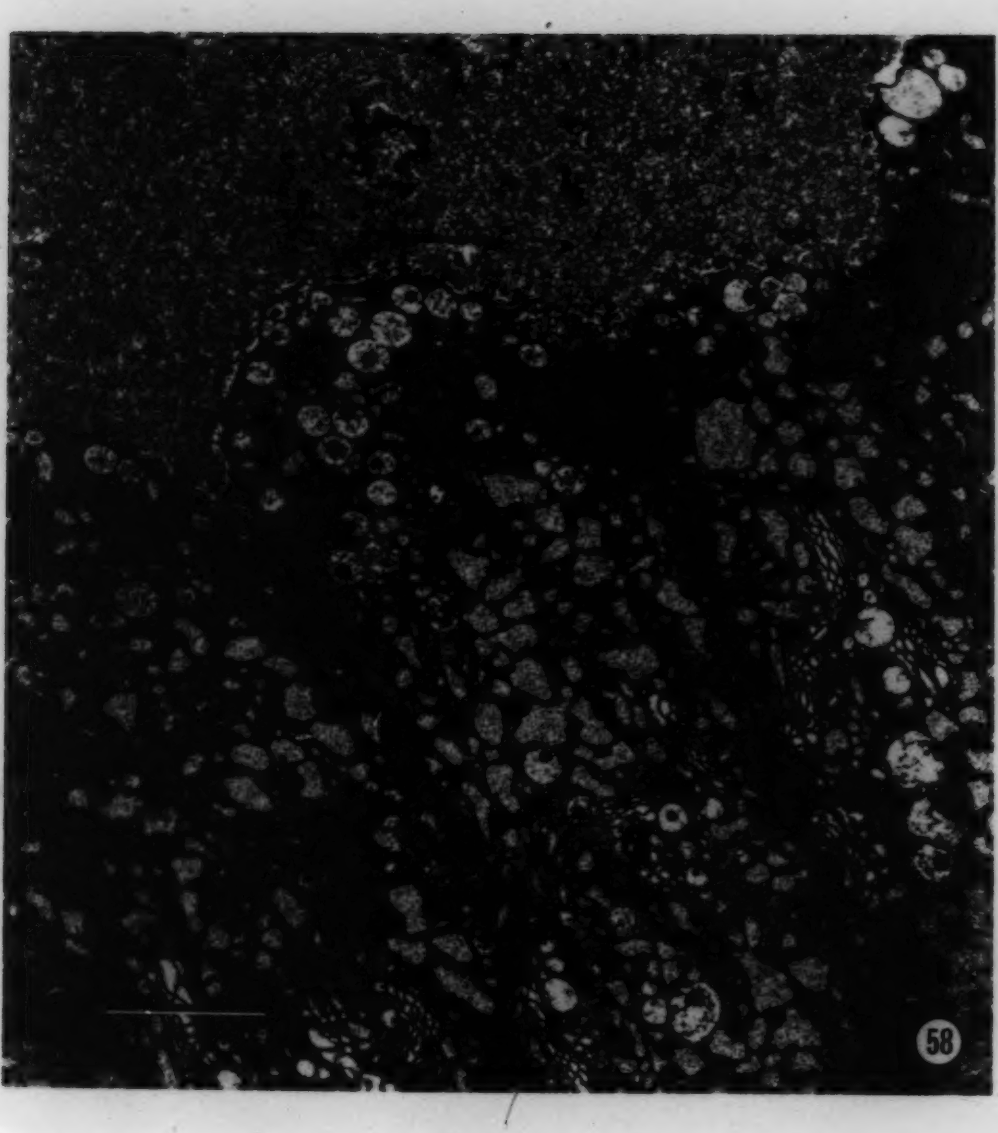

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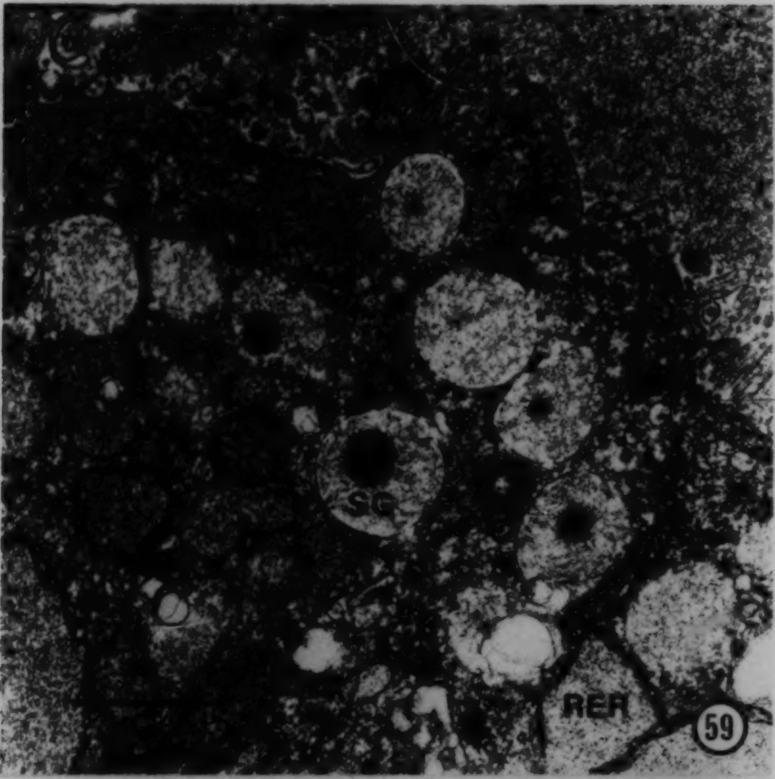
Fig. IV-59. Dorsal prostate-pinealectomy, short days: Apical portion of the secretory cell. A number of secretory granules (SG) and dilated cisternae of rough endoplasmic reticulum (RER) are seen in this portion of the cell. A centriole (C) in cross section is also present in the apical cytoplasm. L, acinar lumen. Bar equals 0.5 μm.

Fig. IV-60. Dorsal prostate-pinealectomy, short days: Apical border of the secretory cell. Note the secretory granules and the apparent exocytosis (arrowhead). L., acinar lumen. Bar equals 0.5 μm.



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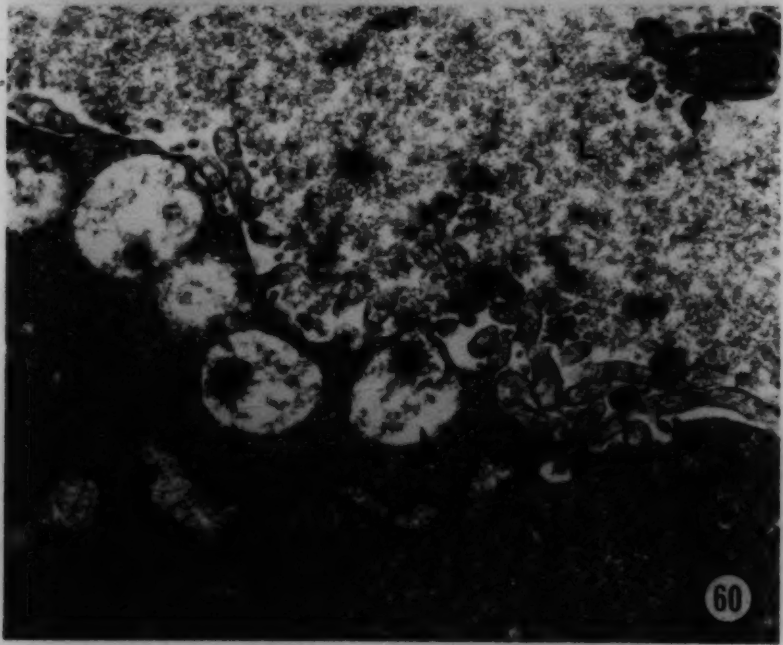

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V- EFFECTS OF SHORT DAYS AND CASTRATION ON THE ACCESSORY SEX GLANDS AND ON LEVELS OF REPRODUCTIVE HORMONES

INTRODUCTION

It is generally believed that short-day photoperiods exert their effects on the gonads and accessory sex glands via the pineal secretion acting through the neuroendocrine-gonadal axis, at the hypothalamic and/or pituitary levels (Berndtson and Desjardins, 1974; Reiter et al., 1980; Jackson et al., 1984; Steger et al., 1984). This leads to a decrease in circulating androgen levels, and a subsequent regression of the accessory sex organs. However, there are occasional studies which suggest a peripheral action of the pineal gland and its hormone melatonin on the male reproductive system (Debeljuk et al., 1970, 1971; Shirama et al, 1981). For instance, Shirama et al. (1981) reported that the reduction in the weights of the accessory sex glands (ventral lobes of the prostate and seminal vesicles) of rats exposed to continuous darkness were not correlated with the plasma gonadotropin and testosterone levels, but these animals showed lower plasma dihydrotestosterone levels as compared with pinealectomized rats exposed to continuous darkness, suggesting a direct effect of the pineal on the accessory sex glands.

In the present study, an attempt was made to examine whether short photoperiod-mediated regression of the accessory sex glands is solely due to a decrease in circulating testosterone levels or whether there are direct influences of short days on the reproductive organs. As both castration and exposure to short days result in a significant reduction in the circulating testosterone levels and weights of the accessory sex glands in male hamsters (Ortiz, 1953; Gaston and Menaker, 1967; Elliott, 1976), castrated hamsters maintained in short days were compared with castrated ones maintained in long days. It was anticipated that, if pineal secretions have any direct effects on the accessory sex glands, then the castrated hamsters maintained in short days might show further regression of accessory sex glands over those castrated ones kept in long days.

The results of the preceding experiments (Chapters II-IV) indicated that the accessory sex glands did not show uniform responses to short photoperiods. For example, the ventral prostate showed the least response to short days. It was therefore of interest to compare the responses of the accessory sex glands to short days and castration, and to see whether short days can induce accessory sex glands regression to the same extent as that induced by castration. To examine this, intact animals maintained in short-day photoperiods were compared with castrated ones kept in long or short day photoperiods for the same period of time.

MATERIALS AND METHODS

In this study, a total of 31 young adult male hamsters was studied (for supply and care of animals, see Chapter II). Twenty-one were castrated via the scrotal route, as outlined below, divided into two groups of 10 and 11 animals, and maintained in long-day or short-day photoperiods (14h:10h L:D, 10h:14h L:D) respectively. The third group of 10 intact hamsters was exposed to the short-day photoperiods. The animals were kept in these lighting conditions for 10 weeks.

Bilateral orchidectomy was performed via bilateral scrotal incisions. The hamster was anesthetized with Metofane and, in the supine position, the skin of the scrotal sac was cleaned thoroughly with 70% ethanol. A small lateral incision of about 1 cm was made through the skin of the scrotum and through the muscular sac. The testis, epididymis, and the distal portion of the spermatic cord, with vas deferens and the afferent and efferent blood vessels of the organs to be excised, were exposed. A single ligature (4-0 silk) was placed around the spermatic cord, and this was severed distal to the ligature, allowing for the removal of the testis and the epididymis. The remaining piece of the spermatic cord and the surrounding fat were returned to the scrotal sac, and the muscle and skin incisions were closed with interrupted vertical mattress sutures using a half-circle cutting FS-2 needle and 4-0 silk. The procedure was repeated on the other side of the scrotum to remove the second testis and epididymis.

After 10 weeks of exposure to the above lighting regime, each animal was weighed and decapitated. Trunk blood was collected in heparinized tubes, and plasma was separated for the analysis of reproductive hormones [testosterone, luteinizing hormone (LH), folicle-stimulating hormone (FSH), and prolactin] as outlined above in Chapter II. The accessory sex glands (ventral and dorsal lobes of the prostate, coagulating glands, seminal vesicles and ampullary glands) were removed and weighed.

Weights of the accessory sex glands and plasma reproductive hormone levels are expressed as mean ± standard error of the mean. The groups were first compared with one-way analysis of variance. Where significant differences were indicated, Student's t-test was then applied to determine groups contributing to these differences. Significance levels were set at $P < 0.05$.

RESULTS

The effects of various treatments on the weights of the reproductive organs, and circulating reproductive hormone levels are depicted in Fig. V-1 and V-2, respectively.

The weights of all of the accessory sex glands in castrated animals maintained in short-day photoperiods did not differ significantly from those of castrated ones kept in long days. However, both groups showed significant reductions in the weights of these organs when compared with those of intact animals maintained in short days.

The plasma levels of testosterone in intact hamsters were significantly higher than those of castrated animals. Although plasma levels of LH in intact hamsters were lower than those of the castrated groups, and the levels of this hormone in castrated hamsters maintained in short days were lower than those of castrated ones kept in long days, the analysis of variance demonstrated that the differences observed between group means were not statistically

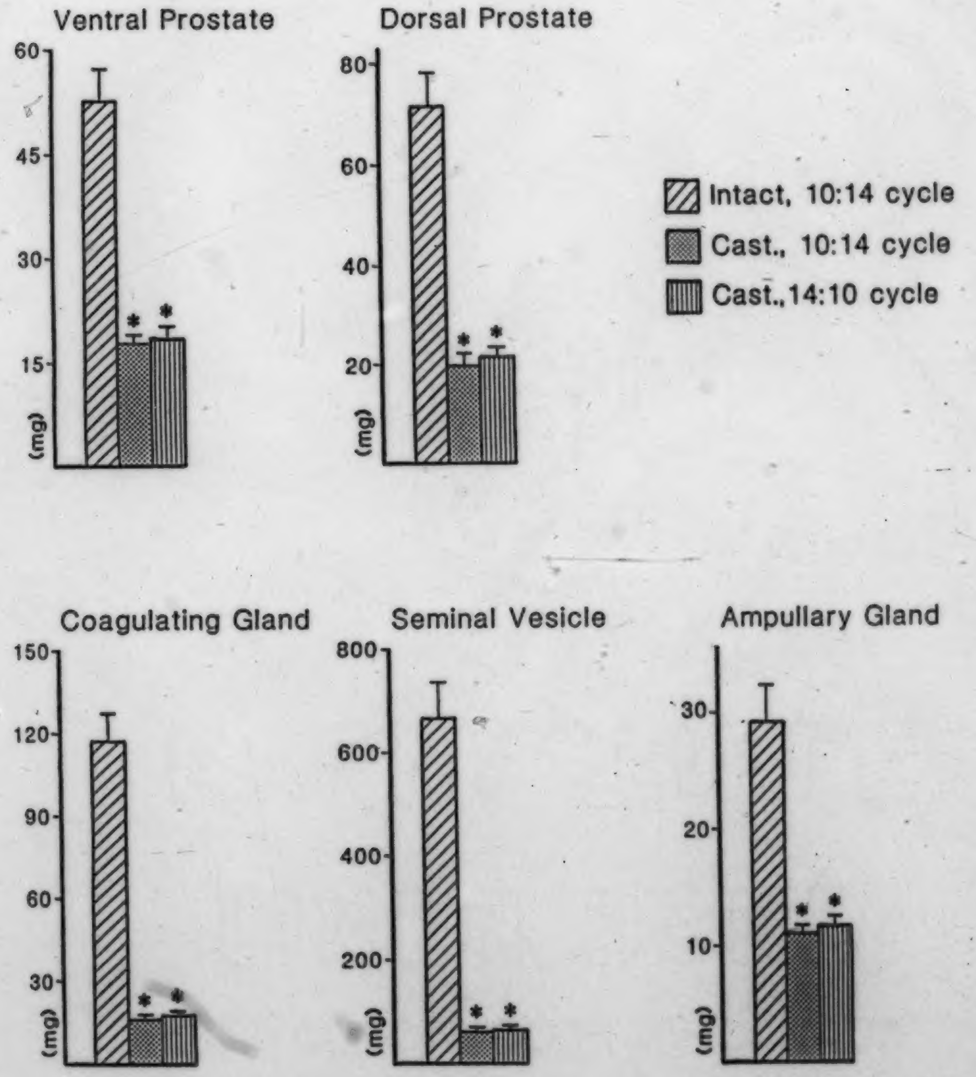

Fig. V-1. Accessory sex organ weights in intact and castrated (Cast.) hamsters kept under either short-day (10:14 cycle) or long-day (14:10 cycle) photoperiods. Data are mean ± standard error of the mean (vertical lines). *$P < 0.05$, *vs.* intact, 10:14. Within castrated animals, there were no significant differences between short and long days.

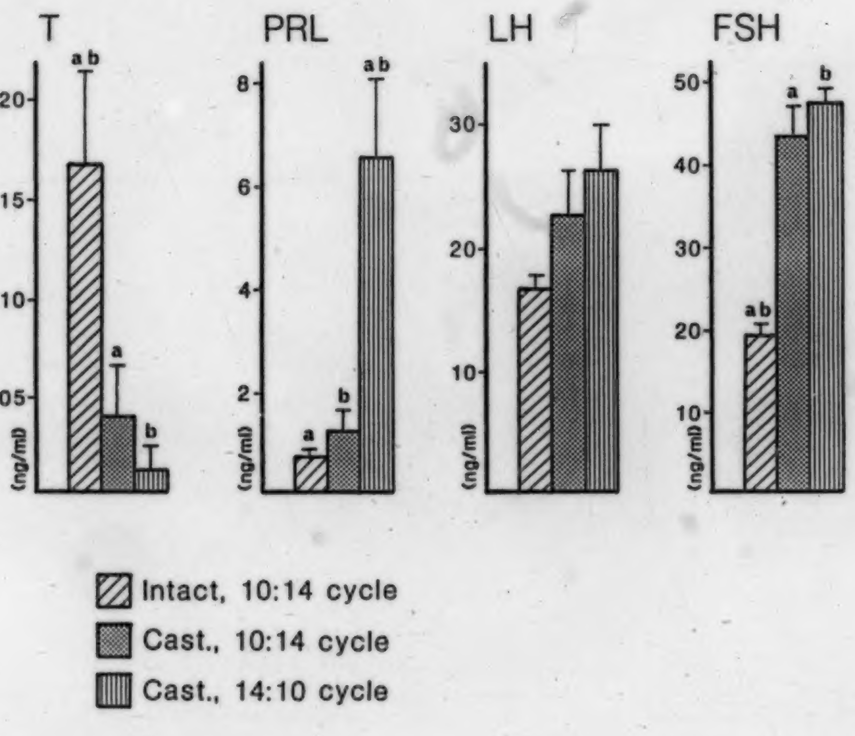

Fig. V-2. Plasma concentrations of reproductive hormones in intact and castrated (Cast.) hamsters kept under either short-day (10:14 cycle) or long-day (14:10 cycle) photoperiods. T, testosterone; PRL, prolactin; LH, luteinizing hormone; FSH, follicle-stimulating hormone. Data are mean ± standard error of the mean (vertical lines). Groups with similar superscripts are significantly different from each other ($P < 0.05$).

significant. On the other hand, plasma FSH levels in intact animals maintained in short days were significantly lower than those of castrated ones maintained in long or short daily photoperiods. The levels of the above hormones in castrated animals kept in short days did not differ significantly from those of castrated ones kept in long days. Short-day exposure of intact or castrated animals resulted in significant reductions in plasma prolactin levels when compared with those of castrated animals kept in long days. Plasma prolactin levels of intact or castrated animals maintained in short days were not significantly different from each other.

DISCUSSION

The results obtained from the present study indicated that, when hamsters were castrated and maintained in long or short daily photoperiods, the weights of their accessory sex glands were significantly lower than those of intact animals kept in short days. The weights of these organs in castrated hamsters did not differ in different photoperiods. From these results, it is possible that short days exposure has no direct effect on the accessory sex glands, as castrated animals maintained in short days did not show further accessory sex glands regression over those castrated ones kept in long days. It is also possible that castration may have induced the maximal regression of these organs which masked any direct influences of short photoperiods.

Lucini et al. (1983) reported that the weights of the prostate glands in hamsters that were castrated and exposed to short days did not differ from those of intact animals kept in the same photoperiod, but the weights of this gland in both of these groups were significantly less than those of castrated hamsters exposed to long days. One possible explanation for the discrepency in the results obtained from the present study and those obtained by Lucini et al., (1983) could be due to the differences in the experimental protocol. In the present study, the animals were castrated on the day they were transfered to short days and were sampled after 10 weeks of exposure to short or long days. In the study by Lucini and co-workers, intact hamsters were first exposed to short or long days for 30 days before half of each group was castrated. Animals were then maintained in their respective lighting regime for an additional 2 weeks. These authors did not indicate which lobe(s) of the prostate was/were examined.

In the present study, the changes in the weights of the accessory sex glands with different treatments correlated well with those of plasma testosterone levels. Both castrated groups had significantly lower plasma testosterone concentrations than did intact animals.

The increase in plasma gonadotropins in castrated animals kept in long or short daily photoperiods is due to the hyperactivity of the hypothalamic-pituitary axis that follows the removal of the negative feedback effect of testosterone (Bardin, 1986). The results of the present study also indicated that plasma LH and FSH levels in castrated hamsters kept in short days did not differ from those of castrated ones kept in long days. These results are in agreement with those reported by Turek (1977) and Ellis and Turek (1979). Turek et al. (1975) reported a decline in serum LH and FSH in castrated hamsters maintained in short days. The differences between the results obtained from the present study and those reported by Turek et al. (1975) may be related to the differences in the experimental design. In Turek's study, the hamsters were castrated after 60 days of exposure to short days whereas, in the present study, the hamsters were castrated and simultaneously transferred from long days to short days. Thus, the response of the hypothalamic-pituitary axis to castration depends on whether the animals were maintained in a stimulatory or non-stimulatory photoperiod prior to castration (Ellis and Turek, 1980).

Plasma prolactin concentrations in intact and castrated groups kept in short days were significantly lower than those of castrated ones kept in long days. These results agree with those reported by Goldman et al. (1981). The decrease in prolactin levels in intact and castrated hamsters maintained in short days could be mediated by the pineal gland as, in the experiment presented in Chapter III, pinealectomy prevented the decline in circulating prolactin levels in hamsters exposed to short-days. The decrease in plasma prolactin levels in castrated hamsters kept in short days appears to be independent of androgenic influence, since the castrated animals kept in long days had higher prolactin values. Goldman et al. (1981) demonstrated that intact, castrated, and castrated hamsters that received exogenous testosterone, all showed a significant decline in serum prolactin levels by 84 days after transfer to short days.

The observations made in the present study indicate that short days exposure could not induce accessory sex glands regression to the same extent as that induced by castration. Furthermore, short days could not induce further regression of the accessory sex glands in castrated animals. This may indicate that the atrophy of the accessory sex glands induced by exposure to short days is primarily due to a depression in the activity of the hypothalamic-pituitary-gonadal axis. This results in a decrease in the synthesis and secretion of testosterone, and the subsequent atrophy of the androgen dependent target organs, such as the accessory sex glands. Nevertheless, in the present study the weights of the accessory sex glands were used as indicators to test the possibility of direct action of the pineal on these organs. This assay might not be sensitive enough to pick up subtle changes. A more detailed study of the physiological consequences of these changes, using biochemical assays of protein and DNA contents, might provide new insights into the mechanisms by which the photoperiod can control the function of the accessory sex glands. This is done in Chapter VII (below).

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VI- EFFECTS OF SHORT DAYS WITH EXCESS PROLACTIN ON THE ACCESSORY SEX GLANDS

INTRODUCTION

The results from the previous experiments indicated that short days exposure of intact hamsters led to a significant reduction in the weights of the reproductive organs. This reduction was accompanied by a substantial drop in circulating testosterone and prolactin levels. Since several studies have suggested that prolactin potentiates the effects of LH on the biosynthesis of testicular androgens (Hafiez et al., 1972) and acts synergistically with testosterone and dihydrotestosterone in promoting accessory sex glands growth (Walvoord et al., 1976; Thompson and Heidger, 1978; Negro-Vilar, 1980), the present study was undertaken to examine whether the presence of excess prolactin could prevent the regression of reproductive organs in hamsters kept in short days.

MATERIALS AND METHODS

To increase circulating prolactin levels, two pituitary glands were implanted under the kidney capsule of the experimental animals. This procedure has been widely used to elevate circulating prolactin levels, as prolactin secretion from the pituitary gland is normally controlled by tonic inhibition mediated by the hypothalamic prolactin release-inhibiting factor (Bishop et al., 1971; McCann and Porter, 1969). Because ectopic pituitaries are separated from hypothalamic influences, the result is an increase in blood prolactin levels (Chen et al., 1970). The isolated pituitary gland secretes prolactin in a pulsatile fasion (Shin and Reifel, 1981). In addition, the studies by Bartke et al. (1982) and Amador et al. (1985) indicate that the effects of hyperprolactinaemia in the male hamster are markedly different from the well documented inhibitory effects of heperprolactinaemia on the pituitary-testicular axis in other species. Amador et al. (1985) reported that adult male hamsters that were given 1/2, 1, 2, 3 and 4 pituitary under the kidney capsule showed no changes in plasma LH or testosterone levels, but LH/hCG and prolactin receptor concentration in the testes gradually increased coincident with increases in plasma prolactin. These elevations were significant with 2 pituitary transplants for prolactin receptors, and with 2 and 3 transplants for LH/hCG receptors.

Eleven young adult hamsters were used in this study (for supply and care of animals, see Chapter II). Each animal received two pituitary glands under the kidney capsule, as outlined below. The pituitary donor animals were female hamsters of the same age as the recipients. One week after the surgery, 5 animals were exposed to short-day photoperiods (10h:14h, L:D), and the remaining 6 animals were maintained in long-day photoperiods (14h:10h, L:D) to serve as controls. All were kept in these lighting conditions for 10 weeks before sacrifice.

To perform pituitary transplantation, the donor hamster was decapitated, and the skin overlying the dorsolateral surface of the skull was removed. Cuts were made through the occipital, parietal, and frontal bones with a bone cutter, and the roof of the skull was removed. The brain was lifted up, and the pituitary gland was removed with fine forceps from the bony fossa, the sella turcica, which is located in the sphenoid bone of the cranium. The recipient hamster was anesthetized with Metofane and placed on its ventral surface. A dorsolateral skin incision was made on the left side near the left costal border of the thorax. The left kidney was approached and exposed by blunt dissection. A small cut, about 2 mm length, was made in the kidney capsule and a small pouch was made under the capsule by inserting the tip of a blunt dissecting probe between the surface of the kidney cortex and the capsule. Two pituitary glands were inserted into this pouch, and the fascia and skin incisions were closed by surgical clips. All animals were allowed to recover from surgery for at least one week before the onset of the experiment.

After 10 weeks of exposure to the above lighting regime, each hamster was decapitated and trunk blood was collected in heparinized tubes for plasma separation. The testes and all the accessory sex glands were removed and weighed. Plasma levels of testosterone, LH, FSH and prolactin were measured in both groups as outlined in Chapter II.

The data were statistically analyzed using Student's t-test. Significance levels were set at $P < 0.05$.

RESULTS

The weights of the accessory sex glands and plasma concentrations of reproductive hormones are illustrated in Fig. VI-1. The presence of pituitary transplants underneath the kidney capsule of hamsters kept under long or short daily photoperiods, resulted in plasma prolactin levels higher than those usually seen in intact hamsters kept in long daily photoperiods. Furthermore, plasma prolactin levels in hamsters maintained in short days were significantly higher than in long days. The weights of the testes, ventral and dorsal lobes of the prostate, coagulating glands and ampullary glands in hyperprolactinemic hamsters kept under short days, were significantly lower than those of hyperprolactinemic animals maintained in long days. The weight of the seminal vesicles in hamsters kept in short days did not differ significantly from that seen in animals kept in long days.

Plasma testosterone levels in hamsters kept in short days, were also significantly lower than those of animals maintained in long days. No significant differences were observed in plasma LH and FSH levels.

DISCUSSION

High prolactin levels in pituitary-grafted hamsters observed in the present study confirms the long-term survival and secretory activity of ectopic pituitary grafts (Chen et al., 1970). It is, however, unclear why higher plasma prolactin concentrations in the present study were detected in animals maintained in short days than

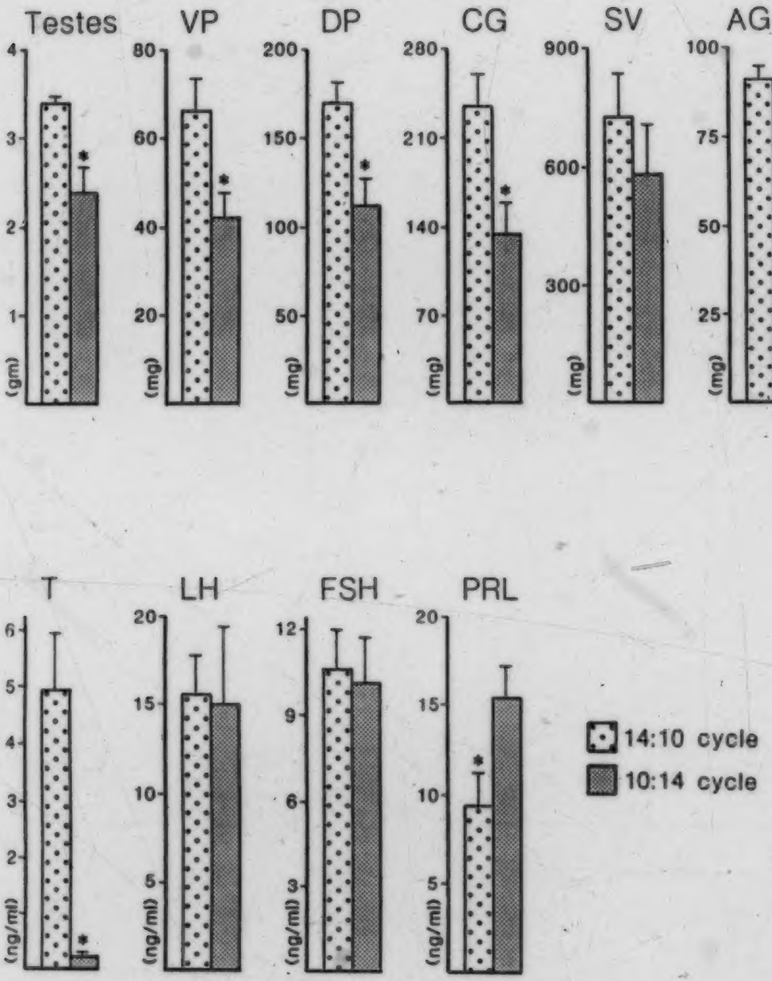

Fig. VI-1. Testicular and accessory sex organ weights and plasma concentrations of reproductive hormones in adult hamsters with pituitary homografts under the kidney capsule, and kept under either long-day (14:10 cycle) or short-day (10:14 cycle) photoperiods. VP, ventral lobes of the prostate; DP, dorsal lobes of the prostate; CG, coagulating glands; SV, seminal vesicles; AG, ampullary glands; T, testosterone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; PRL, prolactin. Data are mean ± standard error of the mean (vertical lines). $^{*}P < 0.05$.

in long days. The present results also indicated that the presence of excess prolactin did not prevent the effects of short daily photoperiods on the weights of the accessory sex glands, except the seminal vesicles. Likewise, serum testosterone levels in hamsters with pituitary grafts kept under short daily photoperiods were lower than those of the corresponding group maintained in long days, whereas plasma LH and FSH levels were similar in both groups of hamsters.

The present data indicate that regression of the accessory sex glands of hamsters kept under short daily photoperiods cannot be explained solely in term of suppressed prolactin release. Chen and Reiter (1980) also demonstrated that neither testicular nor accessory sex organ (combined weights of seminal vesicles and coagulating glands) regression was prevented in blind hamsters that received two pituitary grafts under the kidney capsule. Nonetheless, the studies by Bex et al. (1978) and Bartke et al. (1980, 1983) indicated that the suppression of testicular and accessory sex gland (seminal vesicles and coagulating glands) weights and circulating testosterone levels induced in hamsters by exposure to short days can be reversed by prolactin-secreting ectopic pituitary transplants. These authors also indicated that if such treatment was started at the time of transfer of hamsters from a long to a short photoperiod, it was only partly effective in counteracting the effects of short days on the weights and functions of reproductive organs. These studies suggest that the responsiveness of the testes and accessory sex glands to elevation of peripheral prolactin levels increases if these organs are already regressed (due to exposure to short days) prior to treatment with prolactin. This may explain why the presence of excess prolactin in the present study could not prevent the atrophy of reproductive organs in hamsters maintained in short days.

The differential response of the accessory sex glands to excess prolactin in the present study could be related to differences in prolactin dependence among these organs. Although specific prolactin binding sites have been demonstrated in ventral, lateral, and dorsal lobes of the prostate, and seminal vesicles of rat (Aragona and Friesen, 1975; Barkey et al., 1977, 1979; Witorsch, 1978, 1981; Thompson et al., 1982), there are many conflicting reports concerning the degree of responsiveness of these organs in intact or castrated rats or mice to circulating prolactin (Chase et al., 1957; Bartke, 1967; Bartke and Lloyd, 1970; Yamanaka et al., 1975; Witorsch, 1978; Holland and Lee, 1980; Keenan et al., 1981). It has been reported that prolactin may act indirectly on the accessory sex glands by increase of the testosterone biosynthesis and secretion from the testes (Hafiez et al., 1972; Bartke et al, 1978), and by synergizing with testosterone and dihydrotestosterone within the target cell (Walvoord et al., 1976; Thompson and Heidger, 1978; Negro-Vilar, 1980). It has also been demonstrated that prolactin may exert some influence on the maintenance of the accessory sex glands which is independent of testosterone (Bartke and Lloyd, 1970). Prolactin has been shown to increase the weights of the ventral and dorsolateral lobes of castrated-adrenalectomized rats, and the weights of the seminal vesicles of mice subjected to a similar treatment (Bartke and

Lloyd, 1970). These observations indicate that prolactin may affect the growth of the accessory sex glands even in the absence of any possible source of androgens, and suggest a direct effect of this hormone on the accessory sex glands. Thus, it is possible that the large seminal vesicles which were found in hamsters maintained in short days, could reflect androgen-independent effects of prolactin secreted by the pituitary grafts, as circulating testosterone levels were significantly reduced in these animals.

It can be concluded from the present data that the presence of excess prolactin did not prevent the effects of short days on the weights of the reproductive organs and circulating testosterone levels and thus, the effects of short days on the accessory sex glands are not solely due to a decrease in circulating prolactin levels. Therefore it is more likely that the decrease in circulating testosterone levels resulting from short-day exposure is the major factor causing the atrophy of the accessory sex glands.

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VII- EFFECTS OF PHOTOPERIOD AND THE PINEAL GLAND ON THE ACCESSORY SEX GLANDS IN THE PRESENCE OF LEVELS OF TESTOSTERONE CAPABLE OF MAINTAINING THE SIZE OF REPRODUCTIVE GLANDS IN CASTRATED HAMSTERS

INTRODUCTION

Previous experiments described in this thesis indicated that exposure of young adult male hamsters to short days resulted in significant reductions in the weights of the gonads and accessory sex glands and in circulating levels of testosterone and prolactin; castration in short days did not induce further reduction over castration in long days; and the presence of high circulating levels of prolactin, released by ectopic pituitary glands, did not prevent the suppressive effects of short days on the reproductive organs. In the present study, an attempt was made to determine whether the presence of levels of testosterone capable of maintaining the normal size of the accessory sex glands in castrated hamsters kept in long days can prevent the suppressive effects of short days on the accessory sex glands. It was anticipated from this experiment that, if short-day induced atrophy of the accessory sex glands is solely due to the inhibitory effects of pineal secretions on the hypothalamic-pituitary-gonadal axis, which results in a decrease in endogenous testosterone synthesis and secretion, then the presence of exogenous testosterone will prevent these effects. On the other hand, if the pineal also acts via other mechanisms, then testosterone by itself will not completely prevent the regression of accessory sex glands which follows the exposure of hamsters to short days.

In my previous experiments, tissue and blood sampling were done only at the end of the experiment. In this study, samplings were made at two week intervals until the end of the tenth week. These intermediate samplings were performed to reveal the temporal pattern of changes in the weights and functional activities of the accessory sex glands, and also the reproductive hormone levels during the 10 weeks of exposure to short days. In addition, to confirm the earlier findings of differential responses of the ventral and dorsal lobes of the prostate to photoperiodic and other manipulations, total deoxyribonucleic acid (DNA) and protein content were determined at a number of time points, to demonstrate some of the biochemical consequences of involution.

The present study included two parts: 1) a pilot study to define the dose of exogenous testosterone required to maintain the normal size of accessory sex glands in castrated animals kept in long days, and 2) the definitive study in which the determined dose of testosterone was used to see whether testosterone can prevent the pineal-mediated regression of accessory sex glands.

MATERIALS AND METHODS

Experiment 1. Pilot Study to Define the Dose of Testosterone Required to Maintain the Normal Size of Accessory Sex Glands in Castrated Hamsters.

Twenty young adult Syrian hamsters were utilized in this study. (for supply and animal care, see Chapter II). They were divided into 4 groups of 5 animals each. Animals in three groups were castrated via bilateral scrotal incisions as outlined in Chapter V, and the animals in the fourth group were left intact. Two of the castrated groups received either 10 or 50 μg testosterone (Sigma Chemical Co.) per day for two weeks via ALZET mini-osmotic pumps as outlined below. The third castrated group and the intact one were left untreated to serve as controls. All groups were maintained under controlled conditions of light (14 h light/day, lights on 0600-2000 h) and temperature (22-25 °C).

Testosterone was delivered via implantable ALZET mini-osmotic pumps (Model 2002; pumping rate, 0.5 μl/h for two weeks) (ALZA Co., Palo Alto, CA). Testosterone was dissolved in polyethylene glycol 600 (Sigma Chemical Co.) warmed to approximately 80 °C, then stored in sealed container at 37 °C for 72 h (Will et al., 1980). Pumps were then filled with the testosterone solution, and implanted under the skin of the back of the hamster immediately after the castration surgery was completed. The amount of testosterone delivered per day was estimated according to the concentration of the steroid in the solvent and the pumping rate of the pump. For instance, to deliver 50 μg of testosterone per day, the concentration of the hormone has to be 50 μg/12 μl of polyethylene glycol, as the total volume of the solution released by ALZET pump model 2002, is 12 μl/day.

After two weeks of treatment, each animal was weighed, and a blood sample was collected by cardiac puncture under Metofane anesthesia. Serum fractions from each animal were stored in two aliquots of 300-600 μl volume at -70 °C until the radioimmunoassay analyses for testosterone and LH were performed as outlined below.

The ventral and dorsal lobes of the prostate, coagulating glands, seminal vesicles and ampullary glands were dissected separately and weighed.

Experiment 2. The Effects of the Pineal Gland on the Accessory Sex Glands in the Presence of Testosterone.

In this study, 175 young adult male Syrian hamsters were used. At the onset of the experiment, the hamsters were randomly divided into 7 groups of 25 animals each. The environments were maintained at 22-25 °C with a photocycle of either long days (14h:10h, L:D, lights on 0600-2000 h) or short days (10h:14h, L:D, lights on 0800-1800 h). The groups were subjected to the following treatments:

Maintained in long days:

(1) Intact. (Intact/LD)

(2) Castrated. (Cast./LD)

(3) Castrated + 50 μg testosterone/day. (Cast./T/LD)

Maintained in short days:

(4) Intact. (Intact/SD)

(5) Castrated. (Cast./SD)

(6) Castrated + 50 μg testosterone/day. (Cast./T/SD)

(7) Castrated + 50 μg testosterone/day + pinealectomy.
(Cast./T/Pinx/SD)

Groups 1,2, and 3 will confirm the effects of castration and the effectiveness of hormone replacement. Group 4 and 5, will confirm the effects of short days and of castration in short days, when compared with group 1 and 2. If the short photoperiods act solely by suppressing the hypothalamic-pituitary-gonadal axis, then group 6 will resemble group 3. If it acts via additional mechanism(s), group 6 will fall between group 3 and 4, and group 7 will resemble group 3. If the latter situation is found to be true, further experiments will be required to test whether the pineal hormones act directly on the accessory sex glands or whether a combination of testosterone and some other hormone, such as prolactin, could prevent the antireproductive effects of short photoperiods.

Metofane anesthesia was used for surgical procedures. Pinealectomy was done according to the method of Hoffman and Reiter (1965b) as outlined in Chapter III, and the animals were allowed to recover for an additional week prior to the onset of the experiment. Castration was performed via bilateral scrotal incisions as outlined in Chapter V. Testosterone was delivered via ALZET mini-osmotic pumps as outlined in experiment 1. The pumps were implanted immediately after the castration surgery was completed. As ALZET pump 2002 deliver for only two weeks, each pump was replaced by a fresh one at the end of every second week until the end of the experiment.

The experiment was carried out over 10 weeks. Five animals from each group were sacrificed every two weeks. Blood samples were collected by cardiac puncture under Metofane anesthesia from each animal on day zero (before castration and pump implantation) and when the animal was sacrificed. Serum was stored in several aliquots of 200-600 µl volume at -70 °C for the radioimmunoassay analysis of testosterone, LH, FSH, and prolactin as outlined below. At sacrifice, the body weight and the weights of the testes (when present), and of all of the accessory sex glands (the ventral and dorsal lobes of the prostate, coagulating glands, seminal vesicles and ampullary glands) were recorded. The coagulating glands and seminal vesicles were weighed after their luminal contents were expressed. The ventral and dorsal lobes of the prostate were immediately frozen on solid carbon dioxide and stored at -70 °C for subsequent quantitation of total DNA and protein per organ, as outlined below.

Radioimmunoassay:

Serum levels of testosterone were determined using the Coat-A-Count Kit supplied by the Diagnostic Product Corporation, Los Angeles, CA., with human-based standards. The samples were assayed according to the procedure outlined in the kit. The radioimmunoassay for LH and FSH were done in the laboratory of Dr. G. Bourne, Department of Physiology, University of Alberta, Edmonton, Alberta. The reagents for the assay were supplied by the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases (NIADDK), with rat LH-RP-2 and rat FSH-RP-2 reference preparations. The radioimmunoassay for prolactin was done in the laboratory of Dr. D.E.

Blask, Department of Anatomy, College of Medicine, University of Arizona, Tucson, Ariz., USA. The prolactin assay was performed according to the procedure developed by D. Rodband, Y. Feldman, and M. Jaffe, National Institute of Health, Bethesda, MD. Serum levels of prolactin were measured using a homologous radioimmunoassay and the values are expressed in terms of a purified hamster prolactin preparation.

Total DNA and protein assay:

To measure the total DNA and protein contents in the ventral and dorsal lobes of the prostate, the glands were dissected free from the surrounding tissue, weighed, immediately frozen on solid carbon dioxide, and stored at -70 °C until assayed. The total DNA and protein contents were measured in intact hamsters kept in long or short daily photoperiods at all time points and, in all other groups that were sampled at weeks 2, 6 and 10.

At 0-4 °C (in the cold room), each tissue sample was minced and placed in 9 tissue volumes of buffer (pH 7.5, adjusted at 22 °C) containing 10 mM Tris, 1.5 mM EDTA, and 10% (v/v) glycerol (see Appendix 2). Tissue was homogenized with a Potter-Elvehjem glass-glass homogenizer.

DNA was measured by a fluorometric method. The method is based on the enhancement of the fluorescence of ethidium bromide on intercalating duplex nucleic acids (Karsten and Wollenberger, 1972; Boer, 1975; Morgan et al., 1979). Chemicals used in this assay were supplied by Sigma Chemical Co. The pH 8 ethidium bromide assay solution contained 5 mM Tris, 0.5 mM EDTA, and 0.5 µg/ml ethidium bromide (see Appendix 2). This solution was made up in a 1 litre quantity and stored at room temperature in a light-proof Repipet Ltd. dispensing container. Calf thymus DNA was used as standard. The concentration of this in the stock standard solution (50 μg/ml of buffer containing 10 mM Tris and 0.1 mM EDTA, pH 8) was determined by spectrophotometry at 1 A_{260} (a solution of 50 μg/ml is assumed to have an absorbance (A) of 1 at 260 nm wavelength). Fresh blanks (pH 8 ethidium bromide assay solution without added samples) and calf thymus DNA working standard were prepared on a daily basis. The working standard DNA solution contained 10 μl of stock calf thymus DNA at 1 A_{260} (0.5 μg DNA) added to 1.2 ml of the pH 8 ethidium bromide assay solution.

DNA measurements for each sample were made in triplicate. In 400 μl polyethylene microtest tubes (BIO-RAD), aliquots of 20 μl of homogenate, containing approximately 150-700 μg of fresh tissue, were incubated at 37 °C for 30 minutes with 10 μl of sodium dodecyl sulfate (0.35% in water), 3 μl of proteinase K type XI (10 mg/ml of 50% glycerol), and 2 μl of ribonuclease type II-A (10 mg/ml of 50% glycerol). After incubation, the whole medium was added to 1.2 ml of the pH 8 ethidium bromide assay solution in 10 X 75 mm borosilicate tubes, gently mixed, and the fluorescence measurements were made on a Gilson Spectra/glo Fluorometer. The fluorometer was set with the excitation wavelength of 520 nm, the emission wavelength at 600 nm, and the fluorescence scale was set at X10. The fluorimeter compartment was thermostated at 22 °C. Thus, having set the pH 8 ethidium bromide assay solution at zero for the blank, the fluorescence value of a standard solution containing 10 μl of calf thymus DNA at 1 A_{260} (0.5 μg DNA) is 70.

To calculate the DNA concentration in the sample, the fluorescence intensity of the sample was first divided by two, as the volume of the sample used in the assay was 20 μl compared to 10 μl volume of the standard. From the fluorescence intensity of the standard (70 units), DNA concentration in the standard (50 μg/ml), and the fluorescence intensity of the sample, the concentration of DNA per 1 ml of sample homogenate was determined. From this and the total volume of the sample homogenate (after taking into consideration the dilution factor used in each assay), total DNA content per organ was determined.

Quantitation of total protein was performed according to the method previously described by Lowry et al. (1951), and modified by Peterson (1981). Bovine serum albumin (BSA), crystallized and lyophilized (Sigma Chemical Co.), was used as standard. A stock solution of BSA, 0.5 mg/ml of deionized water, was prepared and stored frozen at -70 °C in aliquots of 10 ml volume. From this stock solution, a series of working standard solutions was prepared twice a week (see Appendix 2) and kept refrigerated at 4 °C.

The tissue homogenate (sample) was diluted 1:40 with deionized water. Aliquots of 200 μl volume of the sample or standard solutions were used for the assay. Each sample was run in triplicate (3 X 200 μl), and the standard in duplicate (2 X 200 μl). In a flint glass disposable culture tube, 13 X 100 mm (Fisher Scientific Co.), the 200 μl sample was brought to a final volume of 1 ml with deionized water. The 200 μl standard solution was made to a final volume of 1 ml by adding 600 μl deionized water and 200 μl of the above buffer diluted 1:40 with deionized water. The diluted buffer was used to avoid the variations between the standard and the sample which also contained the same concentration of buffer. The series of standard solutions also contained 2 blank solutions, each consisting of 200 μl of buffer diluted 1:40, and 800 μl deionized water.

To each sample and standard tube was added 1 ml of solution A (Appendix 2), vortexed and allowed to stand at room temperature for 10 minutes. After 10 minutes, 0.5 ml of solution B was added to each tube, vortexed immediately and allowed to stand at room temperature for a further 30 minutes. The absorbance of the samples and standard solutions was read at 750 nm wavelength with a Gilford Spectrophotometer, Model 260, using disposable plastic cuvets with 4.5 ml capacity (Fisher Scientific Co.).

The concentration of the protein in 1 ml of the sample was calculated by using the appropriate portion of Scatchard Computer Program for IBM pc (The program was provided by Dr. W.A. McBlain, Hormone Receptor laboratory, Department of Medicine). From this and from the total volume of the sample or tissue homogenate, total protein content per organ was determined.

Statistics:

The results are expressed as mean ± standard error of the mean (SEM). Data were analyzed for significance using Student's t-test or by one way analysis of variance (ANOVA) where more than two means were considered. When the treatments or their interactions were found to have significant effect by ANOVA, Students's t-test or the Student-Neuman-Keuls multiple comparison test was applied to determine which groups contribute to the difference. Significance levels were set at $P < 0.05$.

In experiment 2, the comparisons between the following groups of animals were considered:

-Intact/LD *vs*. Cast./LD, Cast./T/LD, Intact/SD, Cast./T/SD, and Cast./T/Pinx/SD.

-Cast./LD *vs*. Intact/LD, Cast./T/LD, and Cast./SD.

-Cast./T/LD *vs*. Intact/LD, Cast./LD, Cast./T/SD, and Cast./T/Pinx/SD.

-Intact/SD *vs*. Intact/LD, Cast./SD, Cast./T/SD, and Cast./T/Pinx/SD.

-Cast./SD *vs*. Cast./LD, Intact/SD, Cast./T/SD, and Cast./T/Pinx/SD.

-Cast./T/SD *vs*. Intact/LD, Cast./T/LD, Intact/SD, Cast./SD, and Cast./T/Pinx/SD.

-Cast./T/Pinx/SD *vs*. Intact/LD, Cast./T/LD, Intact/SD, Cast./SD, and Cast./T/SD.

RESULTS

Experiment 1. Pilot Study to Define the Dose of Testosterone Required to Maintain the Normal Size of the Accessory Sex Glands in Castrated Hamsters:

The results of this experiment are shown in Figs. VII-1 and VII-2. Castration resulted in a significant decrease in the weights of all of the accessory sex glands, compared with intact or castrated hamsters which received 50 μg testosterone per day. In animals which received 10 μg testosterone per day, the weights of all of the accessory sex glands, except the ventral lobes of the prostate, were significantly lower than those of intact animals or ones which received 50 μg testosterone per day. Nonetheless, administration of 10 μg testosterone per day in castrated animals resulted in a significantly higher weights of all of the accessory sex glands, except the ventral lobes of the prostate, when compared with those of castrated animals. Hamsters which received 50 μg testosterone per day showed weights of accessory sex glands which did not differ significantly from those of intact animals.

Serum testosterone levels in castrated hamsters were significantly lower than those of intact or castrated ones which received 10 or 50 μg testosterone per day. Serum testosterone values in hamsters that received 10 μg testosterone per day, were significantly lower than those of intact or castrated ones which received 50 μg testosterone per day. On the other hand, serum testosterone values in hamsters that received 50 μg testosterone per day did not differ significantly from those of intact animals. Serum LH levels in hamsters that were castrated or castrated and received 10 μg testosterone per day were significantly higher from those of intact animals, whereas LH values in hamsters which received 50 μg testosterone per day did not differ significantly than those of intact animals, but were significantly lower from those of untreated castrates.

Experiment 2. The Effects of the Pineal Gland on the Accessory Sex Glands in the Presence of Testosterone.

Reproductive organ weights:

The weights of the testes in intact hamsters kept in long or short days are presented in Fig. VII-3. Intact hamsters maintained in short days (Intact/SD) showed a significant reduction in the weights of the testes at week 8 of the experiment, compared with those of intact ones kept in long days (Intact/LD).

The effects of various treatments on the weights of the accessory sex glands, ventral and dorsal lobes of the prostate, coagulating glands, seminal vesicles and ampullary glands are depicted in Figs. VII-4, 5, 6, 7, and 8 respectively. Two weeks following the onset of this experiment, the castrated animals maintained in either long-day (Cast./LD) or short-day (Cast./SD) photoperiods exhibited a significant reduction in the weights of all of the accessory sex glands except those of the ventral prostate, when compared with those of intact hamsters or castrated ones that received 50 μg testosterone per day and were maintained in the same photoperiod. The weight of the ventral prostate was found to be significantly reduced only after 6 weeks. The weights of the accessory sex glands in the Cast./SD group did not show further regression over those of Cast./LD group throughout the entire time of the experiment.

After 6 to 8 weeks of exposure to short days, the Intact/SD group of hamsters showed a significant reduction in the weights of all of the accessory sex glands, compared to those of the Intact/LD group, Cast./T/SD and Cast./T/Pinx/SD groups except the weight of the ventral prostate which was found to be significantly reduced only after 10 weeks of short days exposure. It was also found that, after 8 weeks of exposure of intact animals to short days, the weights of the dorsal lobes of the prostate and ampullary glands were no longer significantly different from those of castrated ones maintained in the same photoperiods (Figs VII-5, 8), whereas the weights of the ventral lobes of the prostate, coagulating glands and seminal vesicles were found to be similar to those of the castrated ones only after 10 weeks of exposure to short days (Figs. VII-4, 6, 7).

The accessory sex gland weights in castrated hamsters that were pinealectomized and/or treated with testosterone and maintained in short days (Cast./T/SD; Cast./T/Pinx/SD) were comparable to those from Intact/LD or Cast./T/LD.

DNA and Protein Content

Ventral lobes of the prostate:

The mean values of total DNA contents (μg/organ) with different treatments, are illustrated in Fig. VII-9. Only the castrated hamsters that were maintained in long or short days showed a significant reduction in the DNA content, compared with other groups maintained in the same photoperiods. This reduction was found after 6 weeks of exposure to long or short days. DNA content in the Cast./SD group did not significantly differ from the Cast./LD group at any time point of sampling. Furthermore, DNA values in the Cast./T/SD and Cast./T/Pinx/SD groups were comparable to those from Intact/LD or the Cast./T/LD groups throughout the entire time of the experiment.

Although the Intact/SD group of hamsters showed a decline in the DNA contents toward the end of the experiment (week 8 and 10), this reduction was statistically insignificant when compared with the Intact/LD group.

The effects of the various treatments on the total protein contents are depicted in Fig. VII-10. The mean values are expressed as mg protein/organ.

A significant reduction in the protein content of the ventral lobes of the prostate was observed at week 6 in the Cast./LD and Cast./SD groups, as compared with intact animals or castrated animals which received testosterone and were maintained in the same photoperiods. Protein values in the Cast./LD and the Cast./SD groups did not significantly differ from each other at any time point.

In the Intact/SD group, a nonsignificant decline in protein content was evident by week 8, and this reduction was statistically significant at week 10, compared to the Intact/LD, Cast./T/SD and Cast./T/Pinx/SD groups. The protein values in the Intact/SD group at this time of the experiment were no longer different from those of castrated animals kept in the same photoperiods.

No significant differences were observed among protein values of Intact/LD, Cast./T/LD, Cast./T/SD, and Cast./T/Pinx/SD groups at any time points of sampling.

Dorsal lobes of the prostate:

The mean values of total DNA content of the dorsal lobes of the prostate with different treatments are illustrated in Fig. VII-11. The values are expressed as μg DNA/organ.

Castrated hamsters, whether maintained in long or short days, showed a gradual decrease in the DNA content at weeks 2 through 10 of the treatment. A significant reduction in the DNA content in these groups was evident by week 2 of the treatment, as compared with the DNA values in all other groups kept in the same photoperiods. The mean DNA values in the Cast./SD group did not significantly differ from that of the Cast./LD group.

At week 2, 4, and 6 of the treatment, the DNA content in the Intact/SD group was similar to that of the Intact/LD group. However, a significant reduction in the DNA content occurred at week 8 of exposure to short days and no further reduction was noticed at week 10. At week 10, the DNA content in the Intact/SD group was also significantly lower than that of the Cast./T/SD and the Cast./T/Pinx/SD groups.

No significant differences were observed among the DNA values obtained from the Intact/LD, Cast./T/LD, Cast./T/SD, and Cast./T/Pinx/SD groups at any time point.

The mean values of protein contents in the dorsal prostates of hamsters subjected to various treatments are illustrated in Fig. VII-12. A significant decline in the protein content was obvious by week 2 in castrated animals maintained in long or short days, compared with all other groups maintained in the same photoperiods. This same pattern occured after week 6. The sampling at week 10 showed no significant differences between the castrated and intact animals kept in short days, as short days exposure by itself resulted in a significant reduction in the protein content in intact animals at this time of the treatment.

The protein content in the dorsal prostate of castrated hamsters kept in short days, did not significantly differ from the corresponding group kept in long days at any time point of the treatment.

In intact animals kept in short days, a significant reduction in the protein content of the dorsal lobes of the prostate occured by week 6, relative to those found in Intact/LD group, Cast./T/SD and Cast./T/Pinx/SD groups. Short days exposure did not significantly affect the protein contents of the Cast./T/SD or the Cast./T/Pinx/SD groups and protein values in these groups resembled those of the Intact/LD or the Cast./T/LD groups.

Serum Reproductive Hormone Levels

Testosterone:

Figure VII-13 illustrates the mean serum testosterone concentrations in hamsters that were sampled at day 0, and in intact hamsters kept under either long or short-daily photoperiods for ten weeks.

Mean serum testosterone levels were initially high at day 0 (2.6 ng/ml) and rose even higher at week 2 in intact animals kept in long days (3.4 ng/ml), but this increase was statistically insignificant. Nonetheless, by week 4, a dramatic decrease in testosterone levels was evident (1.1 ng/ml) and, a further reduction was noticed at week 6 (0.9 ng/ml) where the levels were sustained through week 10. The same general pattern of early rise and subsequent fall in serum testosterone levels was observed in intact animals held on short days, with the exception of a steady decrease in testosterone levels through week 10 of exposure to short days. At weeks 8 and 10, mean serum testosterone levels in Intact/SD group were significantly lower than the average levels in Intact/LD group (Fig. VII-14).

The effects of various treatments on serum testosterone levels from week 2-10 are illustrated in Fig. VII-14. In cast./LD group, serum testosterone levels were significantly lower than those of Intact/LD at all time points and, than those of Cast./T/LD at week 6 through 10 of the experiment. Testosterone levels in Cast./SD group were significantly lower than those of Intact/SD at week 2 through 6, and than those of Cast./T/SD and Cast./T/Pinx/SD at week 4 through

10. In fact, by week 4, serum testosterone levels in most of the castrated hamsters were undetectable in both photoperiods.

In hamsters maintained in long days, the Cast./T/LD group had serum testosterone levels that were significantly lower than those of intact ones at week 2 of the treatment, but no significant differences were observed at week 4 through 10. Likewise, in hamsters that were held in short days, the Cast./T/SD and the Cast./T/Pinx/SD groups had significantly lower testosterone values at week 2, compared with intact ones, but no significant differences were observed at week 4 and 6. However, as exposure of intact hamsters to short days resulted in a significant decline in serum testosterone levels at week 8 and 10, at these two points of sampling, mean serum testosterone values in the Intact/SD group did not significantly differ from the Cast./SD, but were significantly lower than the average values in the Cast./T/SD and the Cast./T/Pinx/SD groups.

No significant differences were observed in serum testosterone levels among the Cast./T/LD, Cast./T/SD, and Cast./T/Pinx/SD groups.

Luteinizing Hormone (LH):

Mean serum LH levels in hamsters that were sampled at day 0, and in intact animals maintained in long or short days for 10 weeks are illustrated in Fig. VII-15.

Mean serum LH concentrations at day 0 was 0.8 ng/ml. In intact animals kept in long days, a slight increase, although not significant, in LH levels occurred at week 2, followed by a slight but steady decline between 2-10 weeks of the experiment. Such upward and downward trends in serum LH levels were not observed in intact hamsters kept in short days. Although mean serum LH levels in intact hamsters kept in short days were consistently lower than those of intact ones held in long days, these differences were statistically insignificant.

Figure VII-16 illustrates the effects of various treatments on serum LH levels in hamsters which were maintained under long or short daily photoperiods.

In castrated untreated hamsters, whether maintained in long or short days, serum LH concentrations were high at all time points, compared with those of all other groups of hamsters maintained on the respective lighting regime. No significant differences were observed between the castrated groups in both photoperiods.

After 4 weeks of exposure to short days, the Cast./T/SD group demonstrated a steady decline in serum LH levels and, by week 6 through 10, serum LH levels in this group were significantly lower than those of the Cast./T/LD group. The Cast./T/SD group also showed significantly lower serum LH values from those of the Cast./T/Pinx/SD group at week 8 and 10 of the treatment. Serum LH values in the Cast./T/Pinx/SD group, on the other hand, did not significantly differ from those of the Intact/LD or the Cast./T/LD group at any time point.

Follicle-Stimulating Hormone (FSH):

The mean serum FSH levels at day 0 and in intact hamsters kept in long and short daily photoperiods, are shown in Fig. VII-17. The mean serum FSH levels at day 0 was about 8.4 ng/ml. This level of FSH remained more or less stable throughout the entire time of the experiment in the intact animals kept in long days. Serum FSH levels in intact animals kept in short days were consistently slightly lower than those of intact ones kept in long daily photoperiods, but these differences in FSH levels were statistically insignificant.

The castrated untreated groups of hamsters, in both photoperiods, showed a significant increase in serum FSH levels by week 2 through 10 of the treatment (Fig. VII-18), as compared to intact animals maintained in the respective photoperiods.

Among the groups of hamsters held on long daily photoperiods, serum FSH levels in the Cast./T/LD group, did not differ from those of castrated untreated ones at week 2, 4 and 6 of the treatment, but were significantly higher than those of intact ones. By week 8 through 10, a significant decline in serum FSH levels was noticed in group Cast./T/LD, and the FSH values here, while no longer different from those of the Intact/LD group, were significantly lower than those in group Cast./LD (Fig. VII-18).

In hamsters maintained in short days, serum FSH levels in the Intact/SD group were significantly lower than those of Cast./T/SD group at week 2 and 4, and also significantly lower than the Cast./SD and Cast./T/Pinx/SD groups at all time points. Serum FSH levels in Cast./T/SD group were significantly lower than those in group Cast./SD at week 4 through 10, and also significantly lower than the Cast./T/Pinx/SD group at week 6 through 10.

Although serum FSH levels in the Cast./T/SD group were usually lower than those of the Cast./T/LD group, no statistically significant differences were observed between these groups, with the exception of significantly lower FSH concentrations in short days at week 6.

Prolactin:

Serum prolactin concentrations at day 0 were 26.9 ng/ml. No apparent changes occurred in prolactin levels in intact animals kept in long days throughout the entire time of the experiment (Fig. VII-19). Subjecting intact hamsters to short days, on the other hand, resulted in a continuous decline in prolactin levels between 2-10 weeks of the treatment. At weeks 6 through 10, serum prolactin levels in the Intact/SD group were significantly lower than those of the Intact/LD group. Serum prolactin values in the Intact/SD group at week 10 were about 5% of those found in the Intact/LD group (Fig. VII-19).

Figure VII-20 shows the effects of various treatments on serum prolactin levels in groups of hamsters maintained under long or short daily photoperiods. Hamsters that were maintained under long daily photoperiods showed no significant changes in serum prolactin levels with different treatments at any time point, except at week 6 serum prolactin levels in Cast./LD were significantly lower than those of the Cast./T/LD group. Short days exposure on the other hand, resulted in a significant reduction in serum prolactin levels in the Cast./T/SD group at week 6 through 10, and in group Cast./SD at week 8 and 10, compared with the Cast./T/Pinx/SD group. Prolactin levels in the Cast./T/SD group were also significantly lower than those of the Intact/LD and Cast./T/LD groups at week 6 through 10, whereas no significant differences were observed between the Cast./T/Pinx/SD, Intact/LD and Cast./T/LD groups (Fig. VII-20). In fact, at week 10 serum prolactin levels in all of the groups maintained in short days, except the pinealectomized one, were significantly lower than those of the corresponding groups kept in long days.

DISCUSSION

The results of the preliminary experiment (Exp. 1), indicated that castration of adult Syrian hamsters for two weeks resulted in a marked reduction in the weights of all of the accessory sex glands. Sustained release of 10 μg testosterone per day via ALZET mini-osmotic pumps partially prevented the atrophy of the accessory sex glands; however, treatment with 50 μg testosterone/day was required to fully maintain the accessory sex organ weights in castrated males. This concentration of testosterone also maintained serum androgen levels approximately equal to those observed in intact hamsters. The results also showed that treatment with 10 μg testosterone/day partially prevented the post-castration rise of serum levels of LH; treatment with 50 μg testosterone/day, on the other hand, suppressed serum LH concentrations to levels similar to those seen in intact hamsters. These results suggest that, in castrated hamsters maintained in long-day photoperiods, a steady release of 50 μg testosterone/day is capable of 1) restoring the levels of circulating testosterone observed in intact hamsters, 2) suppressing serum LH to intact male levels, and 3) fully maintaining the normal weights of the accessory sex glands. Therefore, this concentration of testosterone was used in the definitive study (Exp. 2).

Testosterone treatment of castrated hamsters kept in long days prevented the post-castration involution of the accessory sex glands, confirming the effectiveness of testosterone replacement. The results also showed that short days could not induce accessory sex glands regression when castrated hamsters were treated with testosterone, as the weights of these organs were similar to those of the corresponding group kept in long days. Two further observations from the present study indicate that the presence of adequate supply of testosterone prevented the atrophic response of the accessory sex glands to short days: 1) the total protein content of the ventral and dorsal lobes of the prostate, and 2) the total DNA content of these glands.

Short-day exposure failed to affect the DNA and protein content of the accessory sex glands in castrated hamsters that were treated with testosterone. This suggests that the atrophy of the accessory sex glands of intact hamsters kept in short days is primarily due to a decrease in circulating testosterone titers which follows exposure to short days.

The present results revealed also the temporal pattern of changes in the weights of the reproductive organs, functional activities of the accessory sex glands, and serum levels of reproductive hormones during the 10 weeks of exposure to long and short daily photoperiods. Intact hamsters kept in short days showed a gradual decline in the weights of the testes from week 2-10 of the experiment. Nonetheless, a significant reduction was observed at week 8 of the treatment. The earlier studies by Hoffman and Reiter (1965a) revealed that a total collapse of the testes was already evident in hamsters kept in short days for only 4 weeks, whereas in a subsequent study, it was found that the treatment period required to achieve the same extent of regression was 7-8 weeks (Reiter, 1968).

As expected from the results of my previous experiments (Chapters II-VI), among the accessory sex glands, the ventral prostate showed the least response to short days. This was evident from the changes observed in the weight, DNA and protein content. In intact hamsters, the weight of this gland was found to be significantly reduced only after 10 weeks of exposure to short days. The dorsal prostate, coagulating glands, seminal vesicles and ampullary glands, on the other hand, showed a steady decline in their weights by week 2 through 10 of the teatment, and a significant reduction after 6 weeks of exposure to short days. According to the present results, it appears that 6-8 weeks of duration is required to induce significnt reproductive organs (testes and accessory sex glands) regression in short days, which is similar to the results described in several previous studies (Reiter, 1968; Berndtson and Desjardins, 1974; Bartke et al. 1981; Steger et al., 1982; Jackson et al., 1984).

The weights, DNA and protein contents of the accessory sex glands in castrated hamsters kept in short days did not differ significantly from those in castrated animals maintained in long days. These results confirm the earlier results presented in Chapter V, indicating that short days could not induce further regression of the accessory sex gland in castrated hamsters.

Both treatments, castration in long or short days, and exposure of intact hamsters to short days, decreased the total DNA and protein content in the ventral and dorsal lobes of the prostate. However, the changes in DNA and protein content in the dorsal lobes of the prostate in response to both treatments were more dramatic than in the ventral prostate, which are in consistency with changes observed in the weights of these glands with both treatments. This also confirms the earlier observations that the ventral prostate is less sensitive to castration or exposure to short days. Nonetheless, the results from all parameters examined (weight, DNA, and protein) in both lobes of the prostate, indicate that the rate of degradation in DNA and protein contents with short-day exposure is slower than with castration. Although both of these treatments induce similar responses, there are temporal differences in the initiation of these responses. It is also apparent from examination of the DNA and protein content in the ventral and dorsal lobes that the decrease in the protein content with both treatments is more rapid than the decline in DNA content.

The male accessory sex glands in mammals are well known for their dependency on androgen for maintenance of their structural and functional integrity (Price and Williams-Ashman, 1961; Brandes, 1966; Tuohimaa, 1980). Removal of the androgenic stimulus by castration induces structural involution and the associated decrease or loss of functional activities (Ortiz, 1953; Butler and Schade, 1958; Brandes, 1966; English et al., 1985; Sugimura et al., 1986a). It has been reported that administration of testosterone in physiological doses to castrated mice and rats restored the weight and functional activities of these organs (Brandes, 1966; Tuohimaa and Niemi, 1968, 1974; Tuohimaa and Soderstrom, 1974). Studies by numerous investigators who used the rat ventral prostate (Heyns et al., 1978; Parker et al., 1978; Pousette et al., 1981; Higgins et al., 1982; Page and Parker, 1982), dorsal prostate, coagulating glands (Bartlett et al., 1984), and seminal vesicles (Higgins et al., 1982), indicated that testosterone regulates the synthesis of several abundant secretory proteins in these glands.

Withdrawal of testosterone, as by castration, has been shown to result in marked metabolic changes in the prostate and seminal vesicles of rats and guinea pigs, leading to rapid tissue involution and loss of cellular constituents such as DNA, RNA, and protein contents (Coffey et al., 1968; Coffey, 1974; Barham et al., 1980; Mariotti et al., 1981; Neubauer et al., 1981; Okamoto et al., 1982; Weinberger, 1984). Treatment of androgen-deficient rodents with exogenous testosterone, on the other hand, stimulated the synthesis of DNA and RNA, and enhanced incorporation of labelled amino acids into protein, and increased the tissue ribosomal population density in these organs (Price and Williams-Ashman, 1961; Liao and Williams-Ashman, 1962; Coffey et al., 1968; Coffey, 1974; Tuohimaa and Niemi, 1974; Williams-Ashman, 1975; Bockrath et al., 1981; Majumdar and Anderson, 1981; Sugimura et al., 1986b). Accordingly, it appears that the reduction in the DNA and protein content in the accessory sex glands of intact hamsters kept in short days and castrated hamsters in both photoperiods, is primarily due to a suppression of circulating testosterone titers which follows these treatments, as treatment of castrated hamsters in both photoperiods with adequate supply of testosterone prevented the degradation of DNA and protein content in these glands.

In the present study, the fall in the total amount of prostatic DNA after castration or short-day exposure of intact hamsters may reflect a decline in the total number of cells due to cell death (Coffey et al., 1968; Barham et al., 1980; Okamoto et al., 1982; Isaacs, 1984). In the study by Isaacs (1984) where the ventral prostate of Copenhagen rats were used, it was shown that testosterone has the agonistic ability to stimulate the proliferation of prostatic cells, and also has antagonistic ability to inhibit prostatic cell death. According to Isaacs, the rapid involution of the prostate following castration is predominantly due to a decreased antagonistic effect of androgen on prostatic cell death.

The present results also showed that serum testosterone levels were higher in hamsters that were sampled at day 0 (before castration and testosterone implantation) and at week 2 in intact animals on both photoperiods, this was followed by a significant decline by week 4 of sampling. The high serum testosterone titers at day 0 and week 2 could be an age related phenomenon. The study by Sisk and Turek (1983) who examined the developmental patterns of serum reproductive hormones in male hamsters, indicated that an increase in serum levels of testosterone occurred between 5-10 weeks of age followed by a decline between weeks 10-14 of age. Hence, the high serum testosterone levels observed in the present study at day 0 and week 2 of the experiment, might be due to pubertal activation of gonadal function as the animals at these two time points of samplings were about 9 and 11 weeks old respectively.

In the present study, serum concentrations of LH and FSH tended to be lower in intact hamsters kept in short days than in long days throughout the duration of the experiment; these differences, however, were insignificant. Although significant reduction in serum gonadotropin levels have been reported in intact hamsters kept in short days (Berndtson and Desjardins, 1974; Turek et al., 1975, 1976; Tamarkin et al., 1976; Goldman and Brown, 1979; Steger et al., 1984), the results of the present study and the previous experiment (see Chapter II), and those reported by other investigators (Reiter, 1975; Bex et al., 1978; Steger et al., 1985) suggest that a decline in levels of circulating gonadotropins does not occur consistently in intact hamsters with short-day exposure.

Castrated hamsters in both photoperiods exhibited the expected post-castration rise in serum LH and FSH levels. The concentrations of these hormones were the same in castrated hamsters maintained in long or short photoperiods, and remained similar throughout the study. These results confirm the earlier results presented in Chapter V, and are in accord with the studies reported by Turek (1977) and Ellis and Turek (1979), indicating that serum levels of gonadotropins in hamsters that were castrated on the day of transfer to short days were similar to those in long days. The increase in serum concentrations of LH and FSH observed in the castrated hamsters in both photoperiods is due to the hyperactivity of the hypothalamic-pituitary axis that followed the removal of negative feedback effect of testosterone. It is well established that the pituitary gonadotropin release in mammals is regulated, in part, by gonadal secretions that act via feedback circuits on the hypothalamic-pituitary axis (Ramirez and McCann, 1965; Gay and Midgley, 1969; Damassa et al., 1976, 1983; Bardin, 1986).

The present data also showed that, at week 2 through 6 of the experiment, serum concentrations of FSH in testosterone-treated castrates maintained in long days were significantly higher than those in the intact controls. At these time points, serum LH levels also tended to be higher in the testosterone-treated castrates. Although it is not clear at this time why there are greater circulating gonadotropins in testosterone treated castrates than in intact controls, one possible explanation for this rise is that, in hamsters at this age, treatment of castrated animals with 50 μg testosterone/day was inadequate to reduce serum concentrations of gonadotropins to levels seen in intact control group, indicated by high serum testosterone values in intact hamsters at this time of the experiment. When serum levels of testosterone in intact animals kept in long days declined and became similar to those of testosterone-treated castrates, no further differences were observed between these groups.

Serum concentrations of LH and FSH in testosterone-treated castrates in short days were consistently lower than the matching group in long days though these differences were not always significant. Serum gonadotropins in short days were often less than

50% of the long-day maintained animals. These results are consistent with those reported by several other laboratories (Tamarkin et al., 1976; Turek, 1977; Ellis and Turek, 1979; Steger et al., 1986). This reduction in serum levels of gonadotropins appears to be dependent on the presence of the pineal gland, as removal of the pineal prevented the suppressive effects of short days in my study. Furthermore, the study by Sisk and Turek (1982) indicated that daily melatonin injections given to testosterone-treated castrated hamsters in long days increased the negative feedback effects of testosterone on gonadotropin release, in a fashion similar to that induced by short-day exposure. It has been suggested that the differential response to testosterone treatment of circulating gonadotropin concentrations in long and short day hamsters, might be due to an increase in the sensitivity of the hypothalamic-pituitary axis to the feedback effects of testosterone in hamsters that are exposed to short days (Tamarkin et al., 1976; Turek, 1977). It has also been suggested that short day-induced changes in gonadotropin secretion are secondary to changes in neurotransmitter turnover and hypothalamic luteinizing hormone-releasing hormone (LHRH) content (Steger et al., 1986). The study by Steger and co-workers (1986) indicated that in testosterone-treated castrated hamsters, short-day exposure led to a reduction in hypothalamic norepinephrine and dopamine metabolism and an increase in hypothalamic LHRH content, which appeared to be related to a decrease in LHRH release.

Plasma levels of prolactin were severely reduced in all groups of hamsters that were maintained in short days, except those which were pinealectomized. These results closely agree with those indicated in the previous experiments (Chapter II, III and V) and those reported by others (Matt and Stetson, 1980; Reiter, 1980; Goldman et al., 1981; Borer et al., 1982; Bartke et al., 1984). The marked reduction in serum prolactin concentrations might be one of the factors that resulted in lower circulating gonadotropin levels in testosterone-treated castrates, since it has been demonstrated that prolactin released by pituitary transplants reduced the ability of exogenous testosterone to suppress LH and FSH release in castrated adult hamsters kept in long or short days (Bartke et al., 1984). Furthermore, Matt et al. (1984) reported that testosterone was more effective in inhibiting LH and FSH release in hamsters that were treated with a prolactin release inhibitor than it was in untreated hamsters. The results of these studies suggest that the suppression of prolactin release in short days might be partially responsible for the increase in the sensitivity of LH and FSH release to inhibitory effects of testosterone.

It can be concluded from the results presented in the present study that short-day exposure results in atrophy of accessory sex glands, and this atrophy is associated with a decrease in DNA and protein content. Although serum prolactin levels are significantly inhibited in short days, treatment with adequate supply of testosterone abolishes the atrophic effects of short days. These results strongly suggest that the observed effects of short days are primarily due to a decrease in circulating testosterone levels, which are mediated by the action of pineal secretions on the hypothalamic-pituitary-gonadal axis.

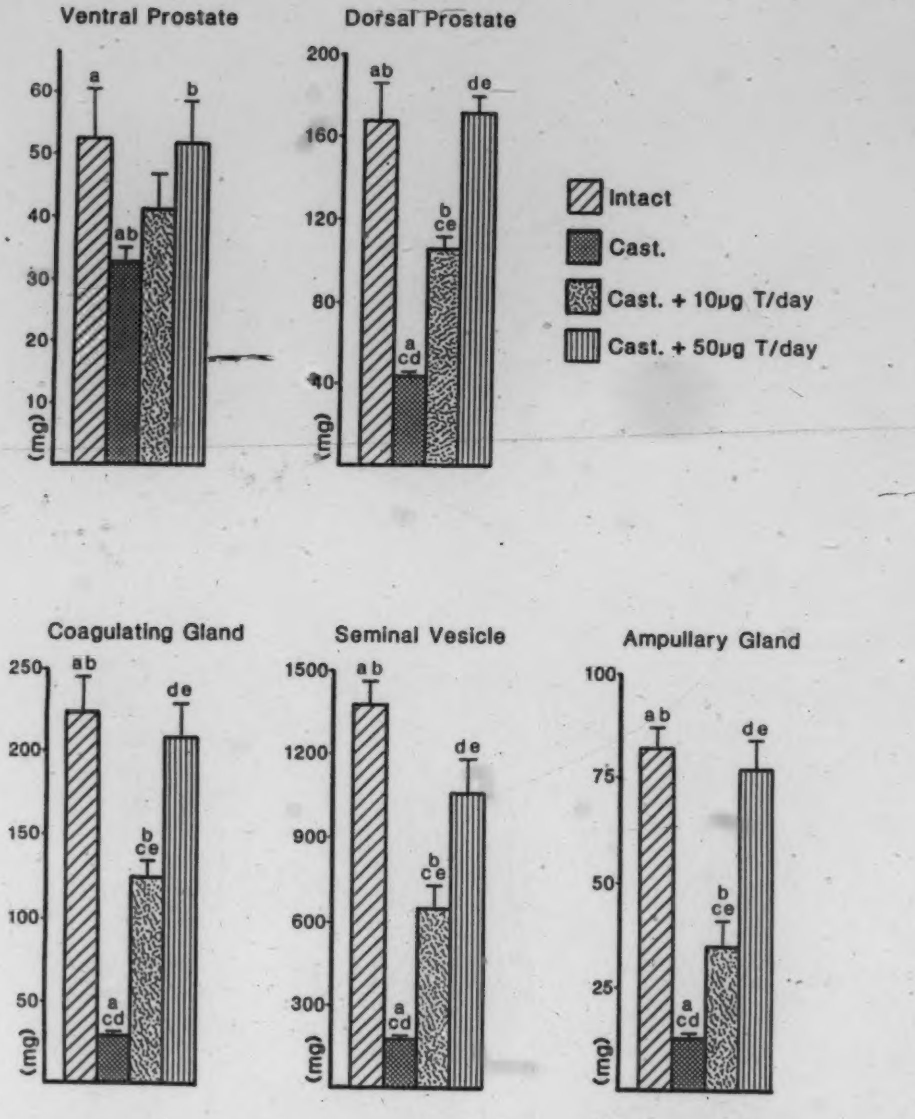

Fig. VII-1. Weights (mean ± SEM) of the accessory sex glands of hamsters that were either left intact or castrated untreated, castrated and treated with 10 or 50 μg testosterone/day via implantable mini-osmotic pumps for two weeks. All animals were maintained under long daily photoperiods (14L:10D cycle). Groups with similar superscripts are significantly different from each other ($P < 0.05$).

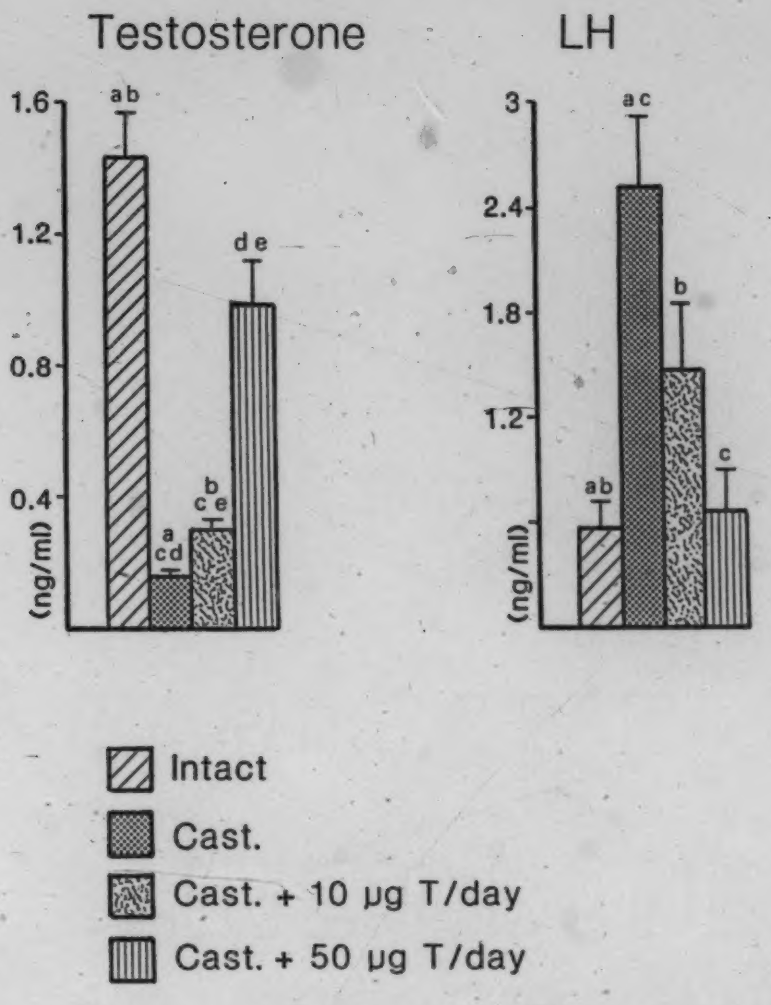

Fig. V11-2. Serum levels (mean ± SEM) of testosterone and luteinizing hormone (LH) in hamsters treated as described in Fig. VII-1. Groups with similar superscripts are significantly different from each other ($P < 0.05$).

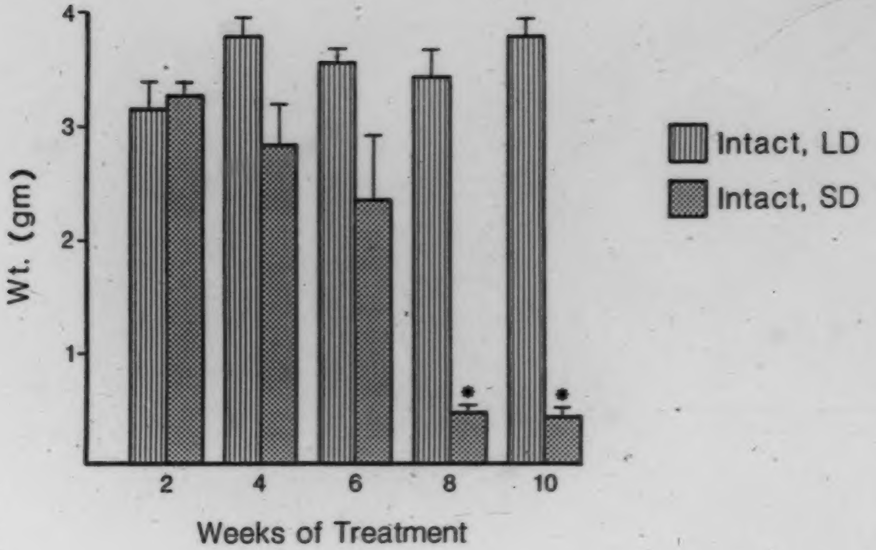

Fig. VII-3. Weights (mean ± SEM) of the testes in intact hamsters maintained under long-day (LD, 14:10 cycle) or short-day (SD, 10:14 cycle) photoperiods. The experiment was carried out over ten weeks, and five animals were sacrificed at two-week intervals. $^{*}P < 0.05$.

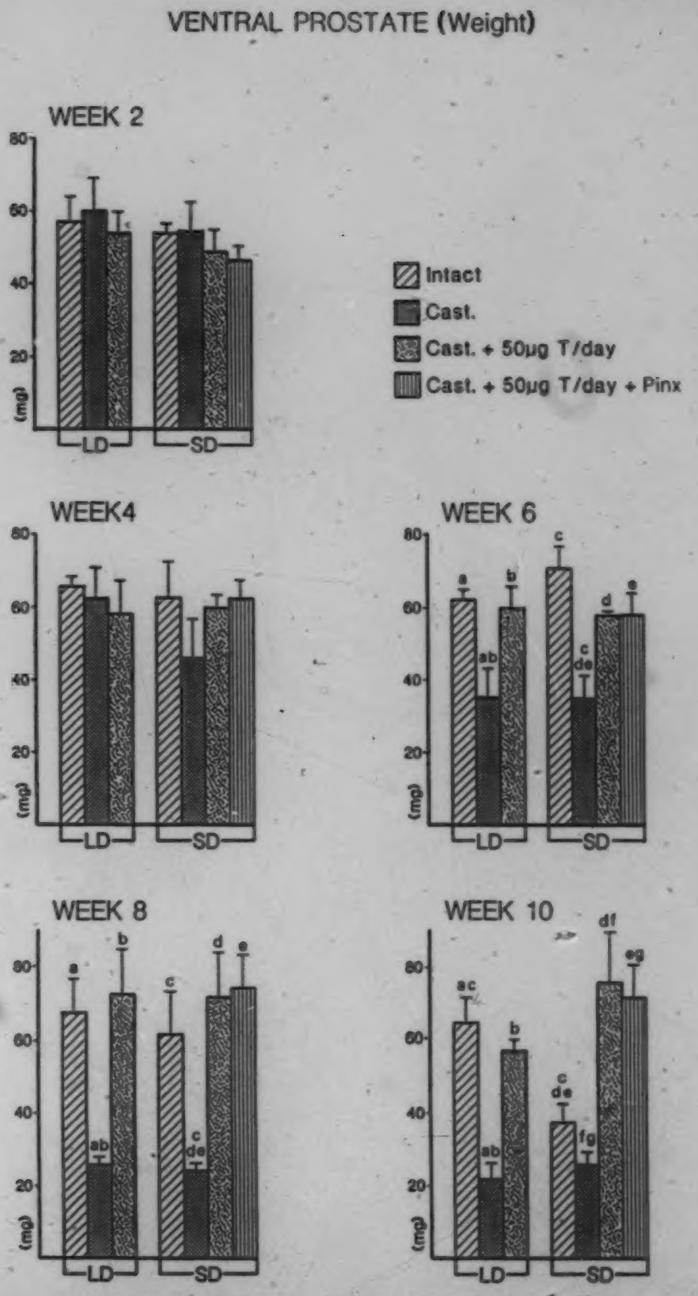

Fig. VII-4. Weights (mean ± SEM) of the ventral lobes of the prostate of hamsters which, after blood sampling on day 0, were either left intact or, castrated (Cast.) untreated, Castrated and given testosterone (T) implants or castrated-pinealectomized (Pinx) and given testosterone implants. The animals were maintained under either long-day (LD) or short-day (SD) photoperiods. The experiment was carried out over ten weeks. Five animals from each group were sacrificed at two-week intervals. Groups with similar superscripts are significantly different from each other ($P < 0.05$).

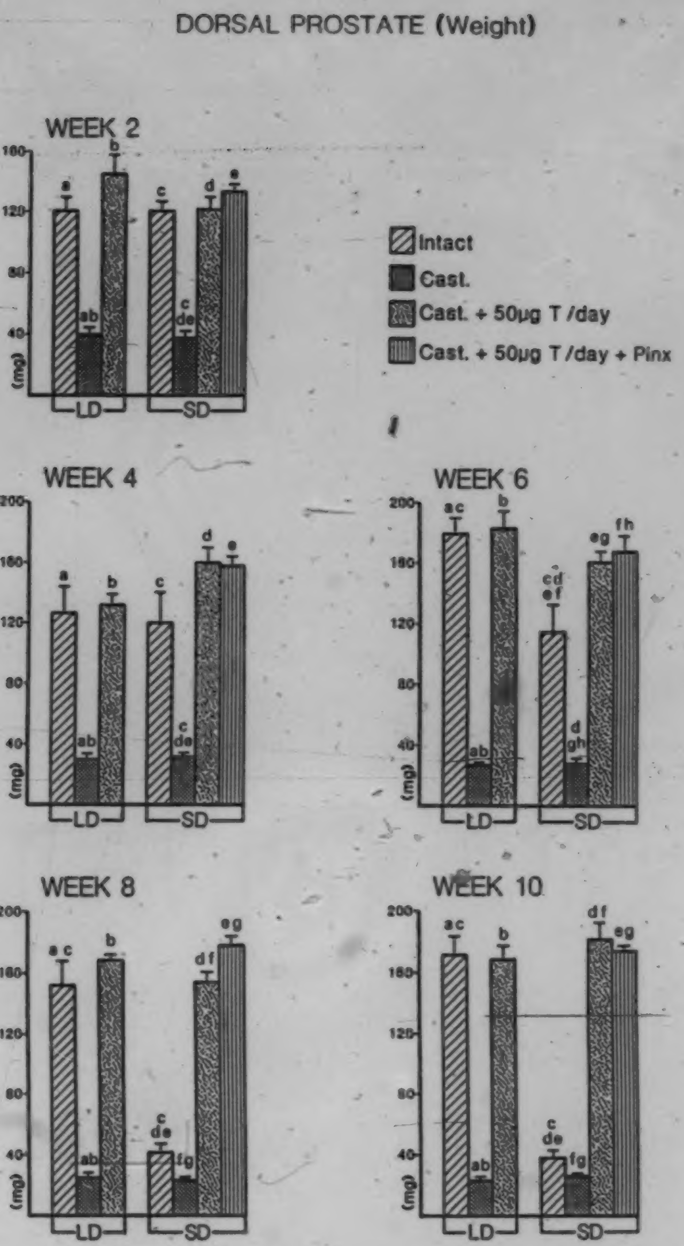

Fig. VII-5. Weights (mean ± SEM) of the dorsal lobes of the prostate of hamsters treated as described in Fig. VII-4. Groups with similar superscripts are significantly different from each other (*$P < 0.05$*).

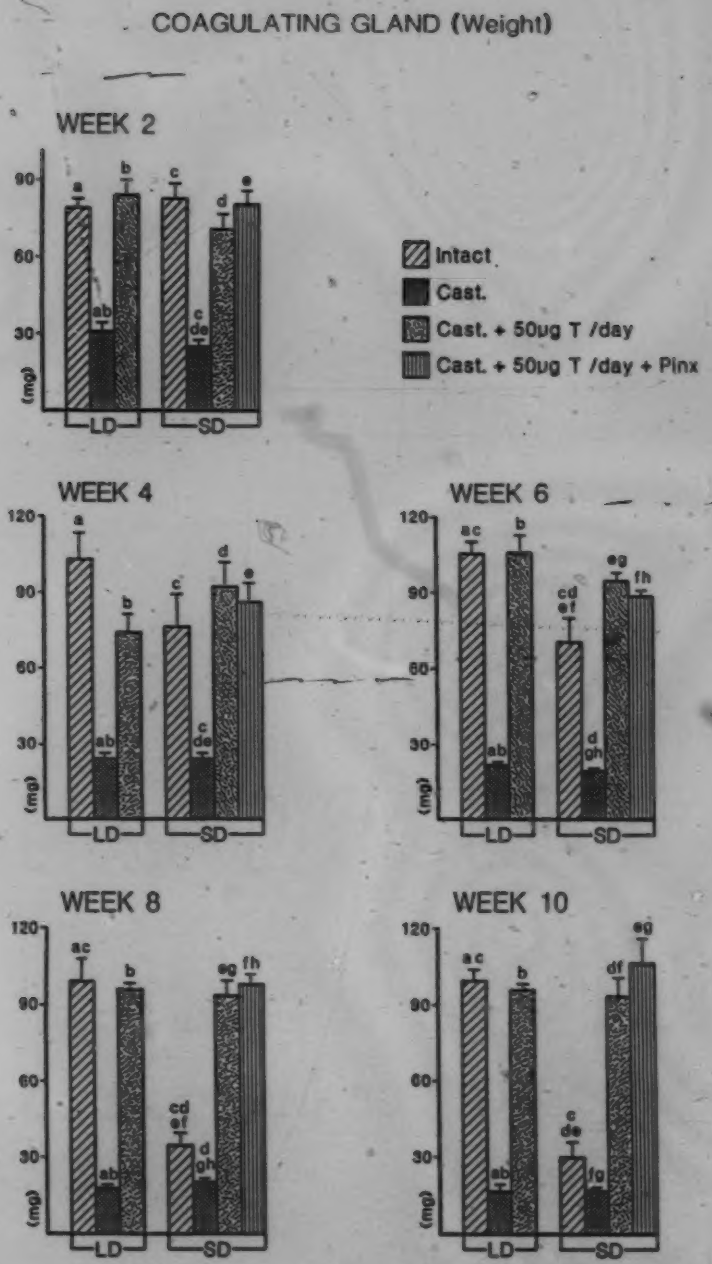

Fig. VII-6. Weights (mean ± SEM) of the coagulating glands of hamsters treated as described in Fig. VII-4. Groups with similar superscripts are significantly different from each other ($P < 0.05$).

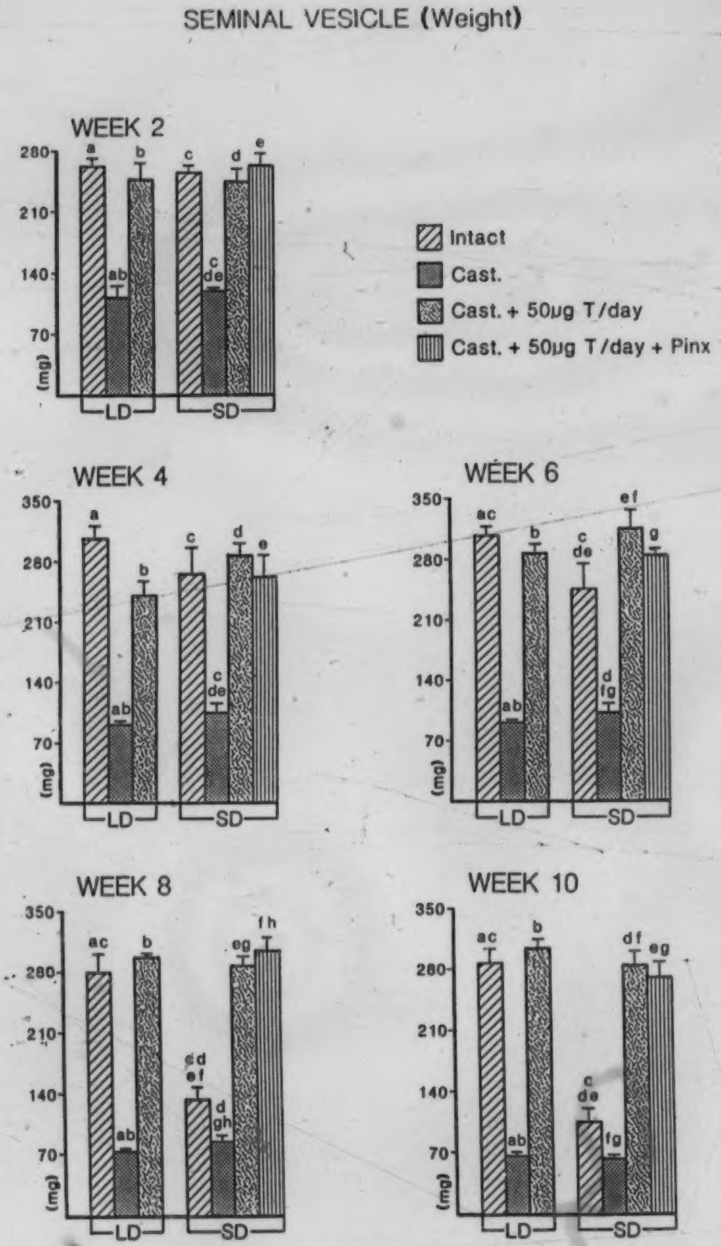

Fig. VII-7. Weights (mean ± SEM) of the seminal vesicles of hamsters treated as described in Fig. VII-4. Groups with similar superscripts are significantly different from each other (*P < 0.05*).

AMPULLARY GLAND (Weight)

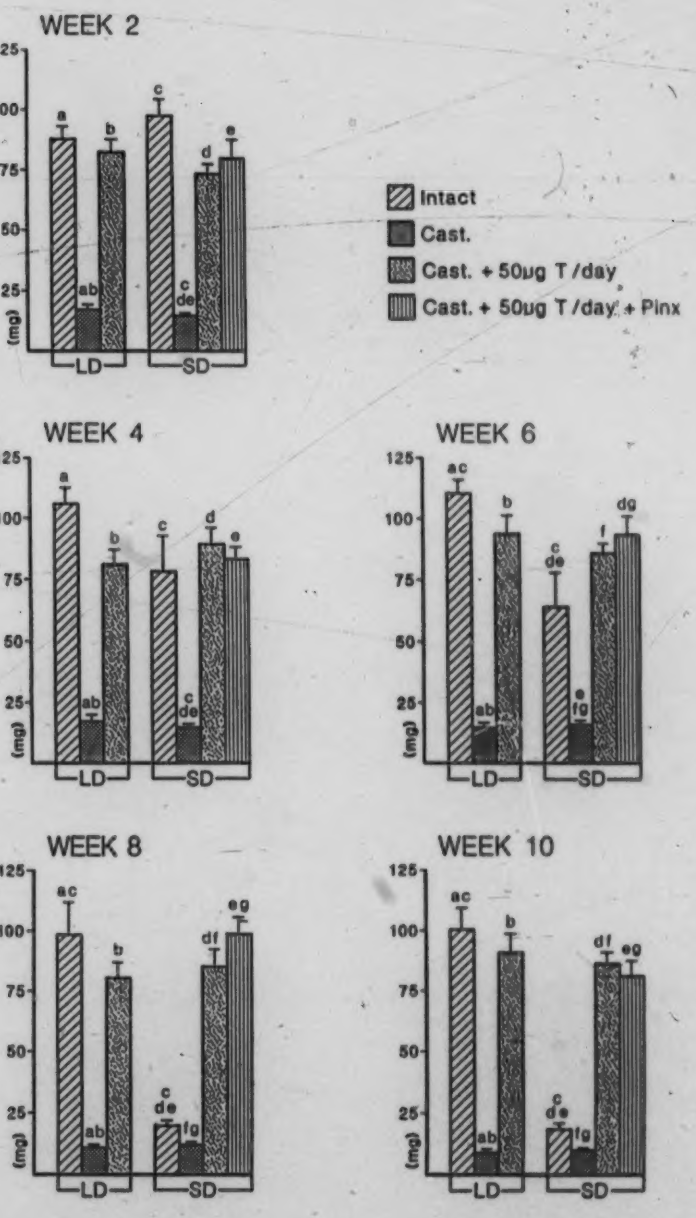

Fig. VII-8. Weights (mean ± SEM) of the ampullary glands of hamsters treated as described in Fig. VII-4. Groups with similar superscripts are significantly different from each other ($P < 0.05$).

VENTRAL PROSTATE (DNA content)

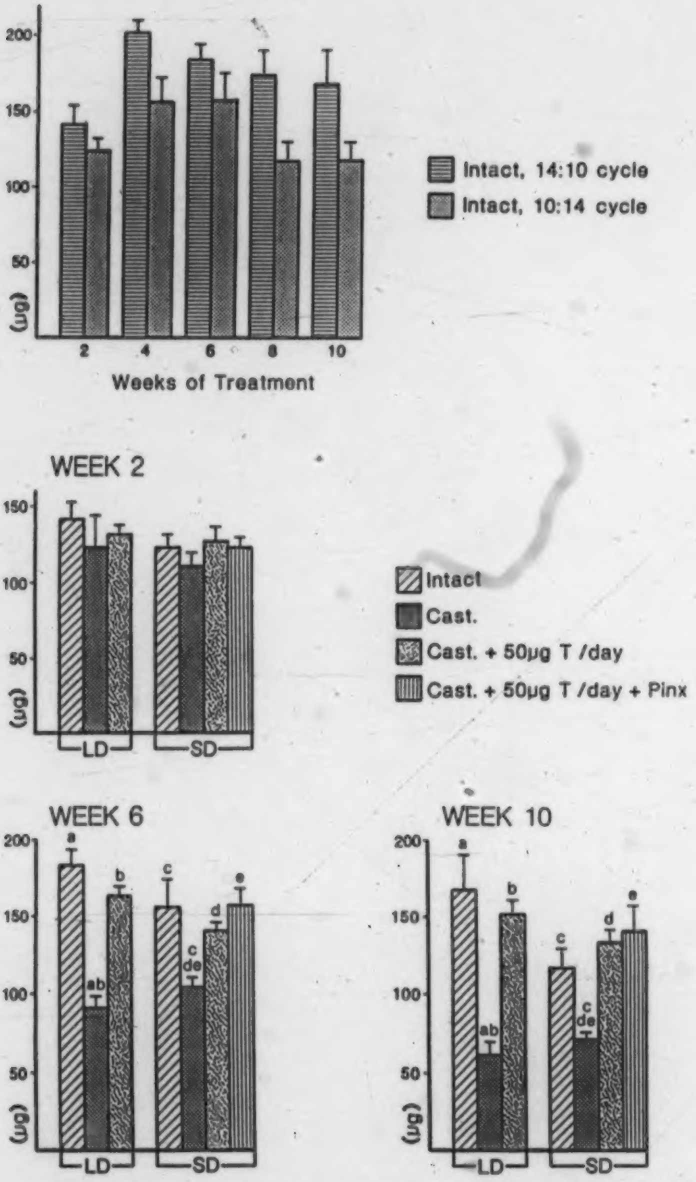

Fig. VII-9. Total DNA content (mean ± SEM) of the ventral lobes of the prostate of hamsters treated as described in Fig. VII-4. Groups with similar superscripts are significantly different from each othre (*P* < *0.05*).

VENTRAL PROSTATE (Protein content)

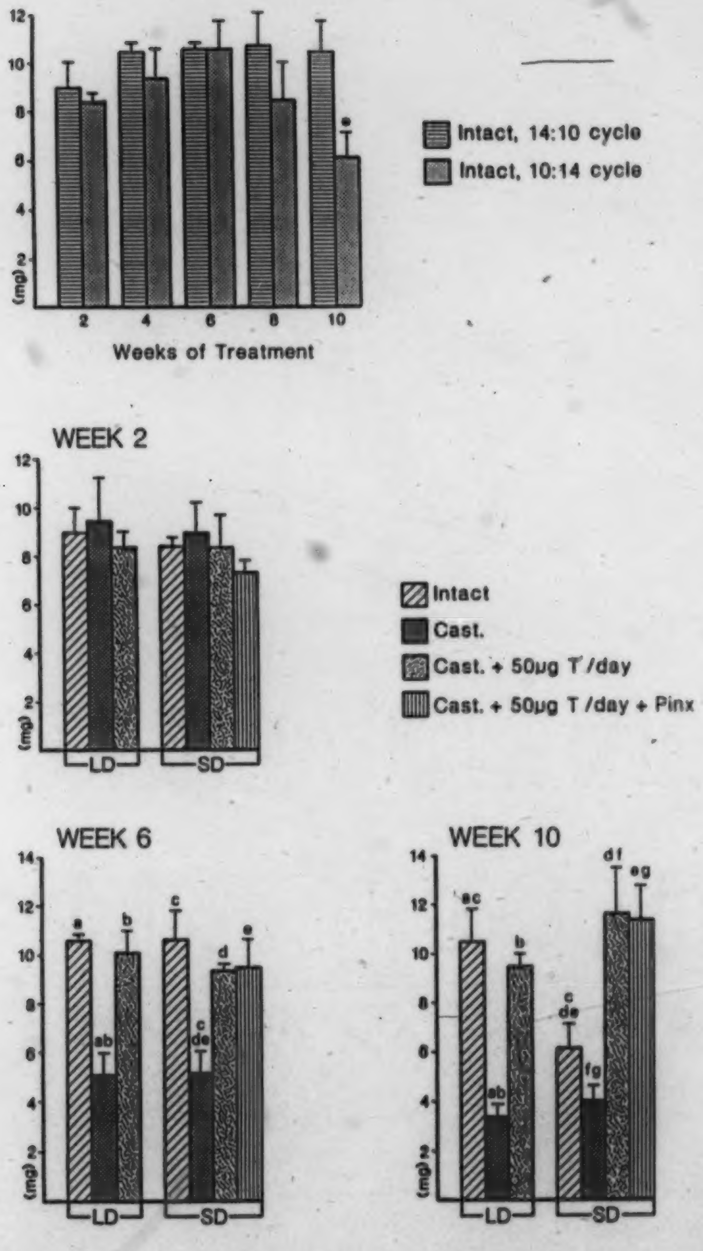

Fig. VII-10. Total protein content (mean ± SEM) of the ventral lobes of the prostate of hamsters treated as described in Fig. VII-4. Groups with similar superscripts are significantly different from each other (*P < 0.05*).

DORSAL PROSTATE (DNA content)

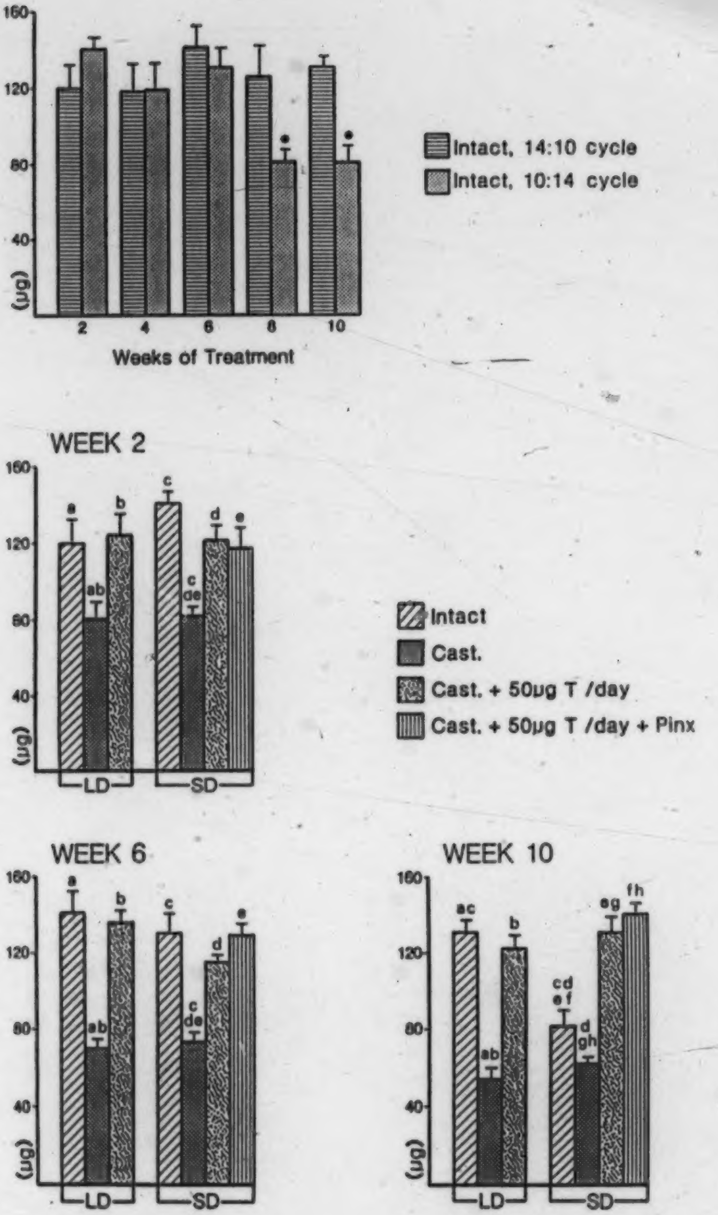

Fig. VII-11. Total DNA content (mean ± SEM) of the dorsal lobes of the prostate of hamsters treated as described in Fig. VII-4. Groups with similar superscripts are significantly different from each other ($P < 0.05$).

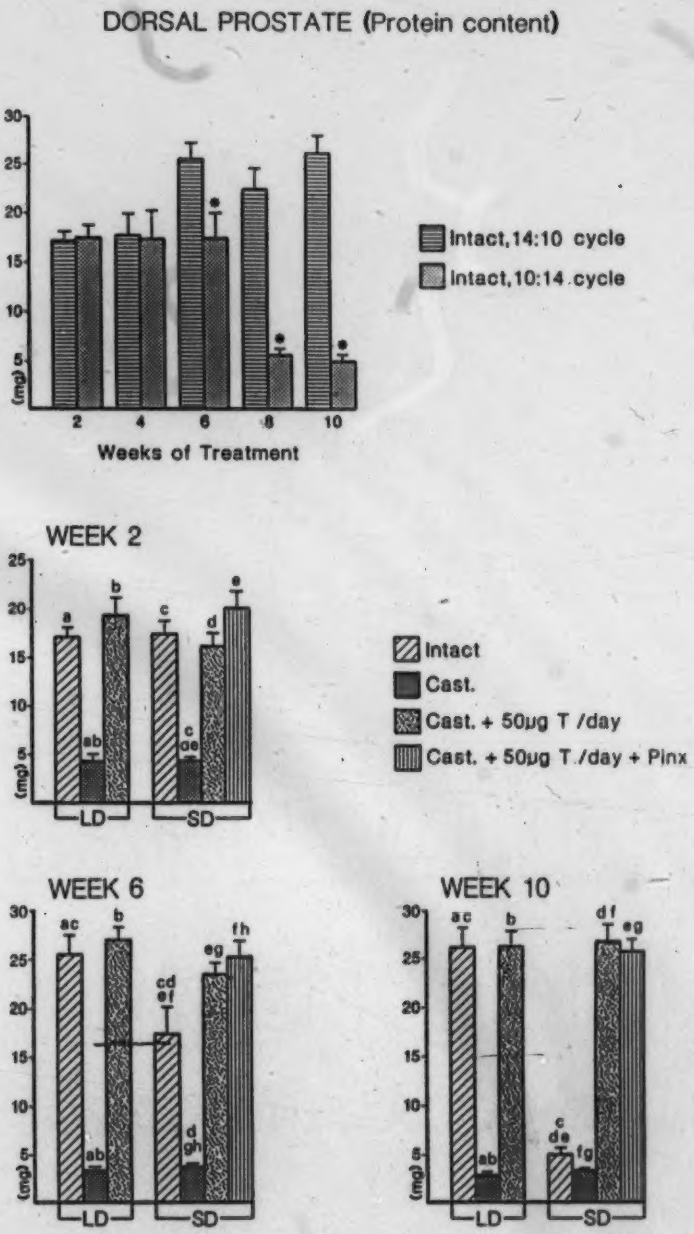

Fig. VII-12. Total protein content (mean ± SEM) of the dorsal prostate in hamsters treated as described in Fig. VII-4. Groups with similar superscripts are significantly different from each other ($P < 0.05$).

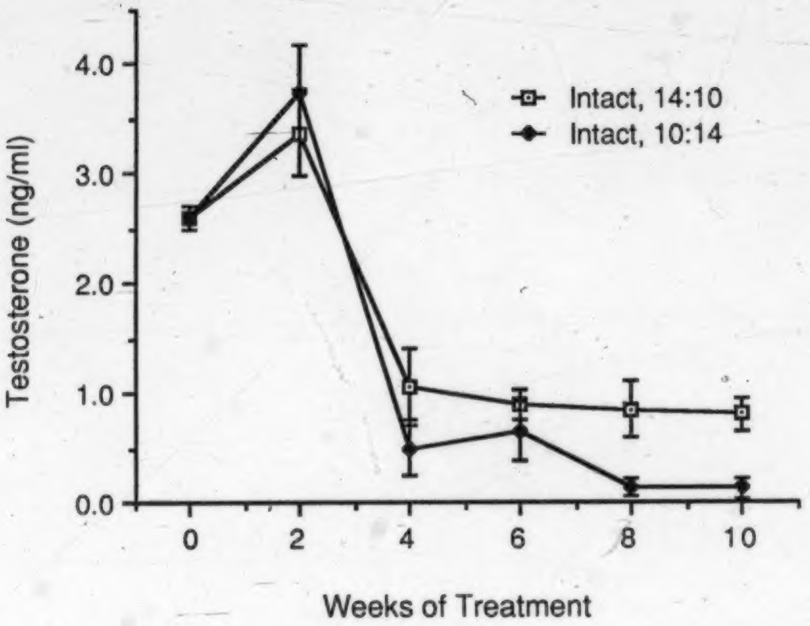

Fig. VII-13. Serum testosterone levels (mean ± SEM) in hamsters that were sampled at day 0, and in intact hamsters maintained in long or short daily photoperiods.

TESTOSTERONE LEVELS

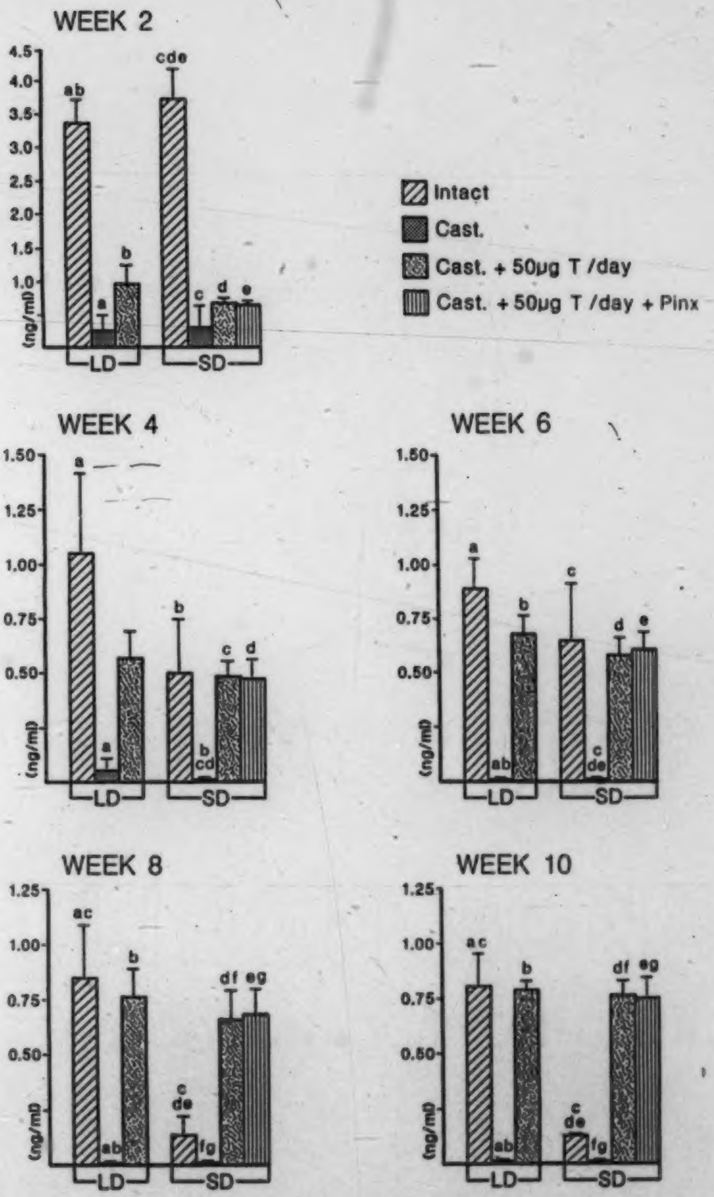

Fig. VII-14. Serum testosterone levels (mean ± SEM) in hamsters treated as described in Fig. VII-4. Groups with similar superscripts are significantly different from each other. ($P < 0.05$).

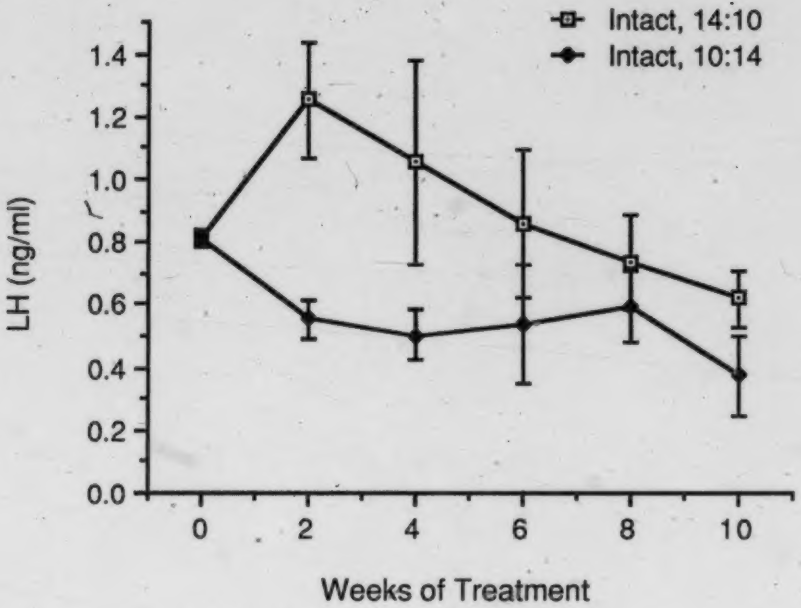

Fig. VII-15. Serum luteinizing hormone levels (mean ± SEM) in hamsters that were sampled at day 0, and in intact hamsters maintained in long or short daily photoperiods.

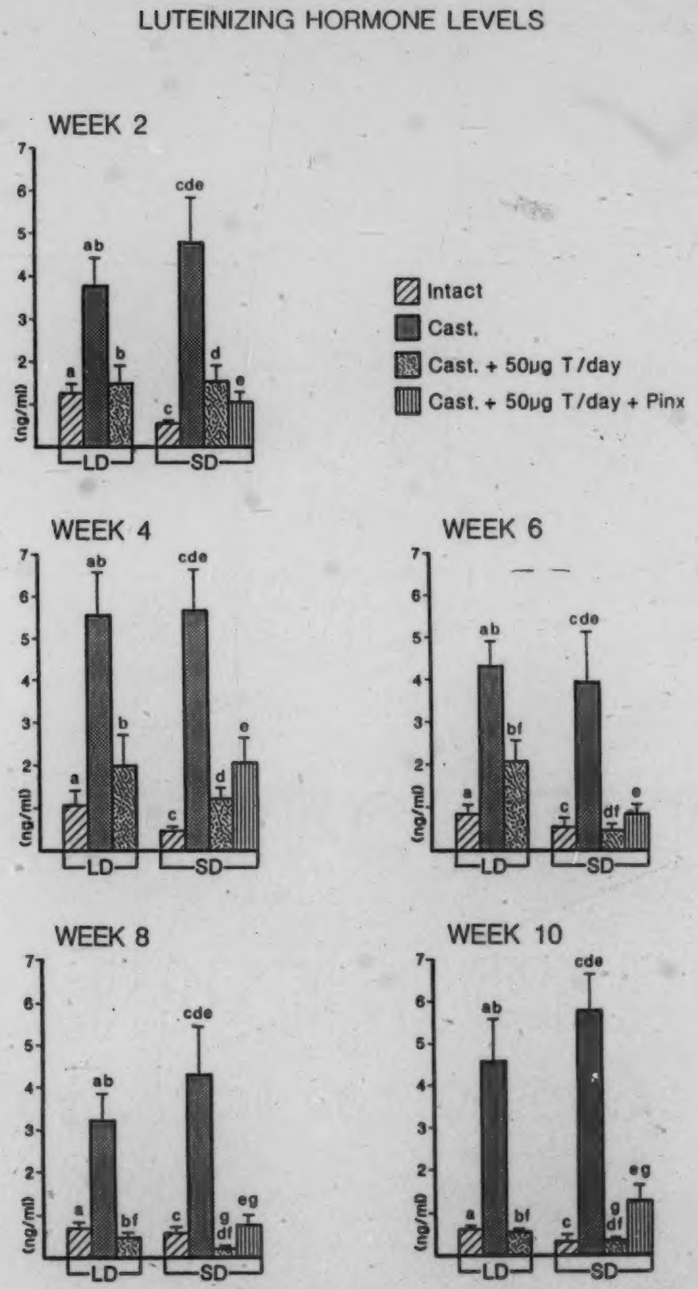

Fig. VII-16. Serum levels (mean ± SEM) of luteinizing hormone in hamsters treated as described in Fig. VII-4. Groups with similar superscripts are significantly different from each other ($P < 0.05$).

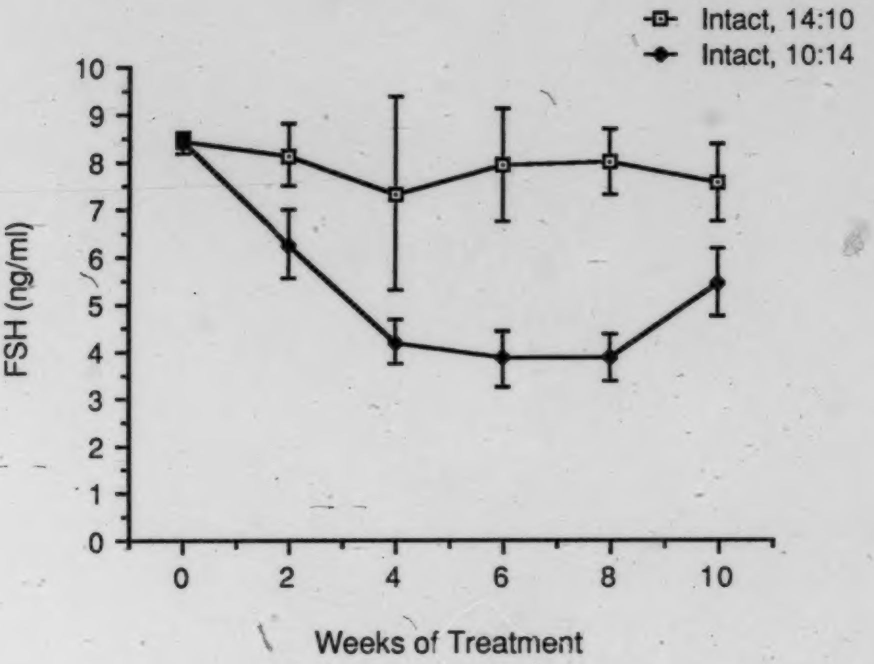

Fig. VII-17. Serum levels (mean ± SEM) of follicle-stimulating hormone in hamsters that were sampled at day 0 and in intact hamsters maintained in long or short daily photoperiods.

FOLLICLE STIMULATING HORMONE LEVELS

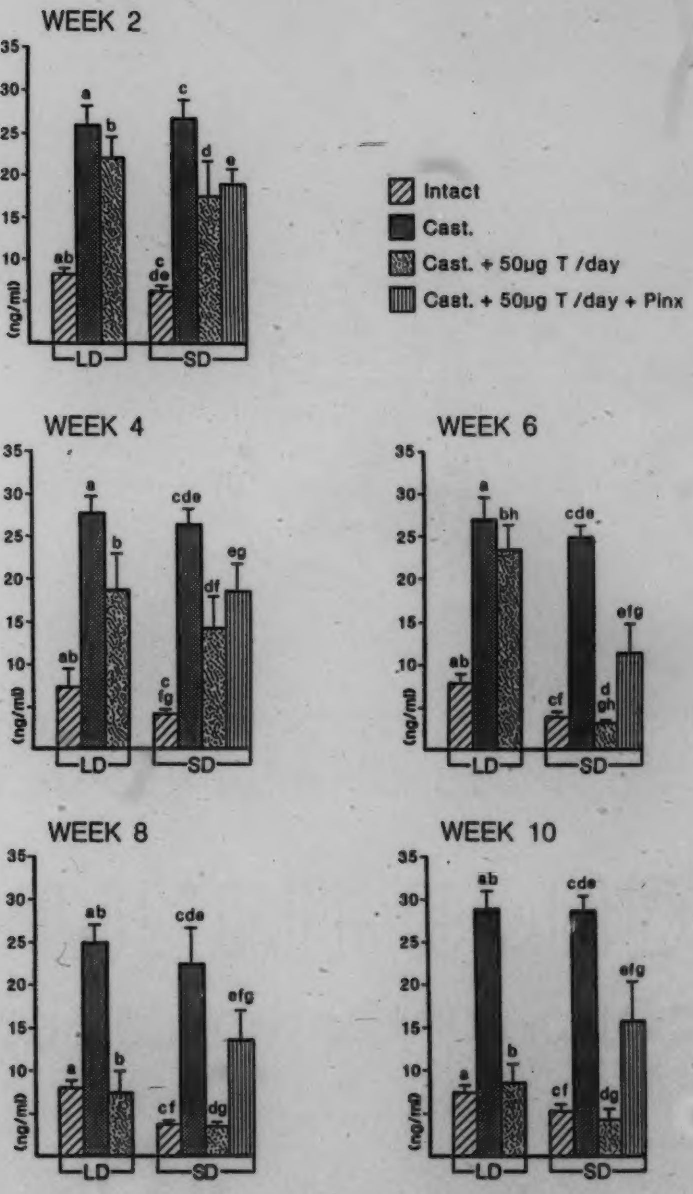

Fig. VII-18. Serum levels (mean ± SEM) of follicle-stimulating hormone in hamsters treated as described in Fig. VII-4. Groups with similar superscripts are significantly different from each other ($P < 0.05$).

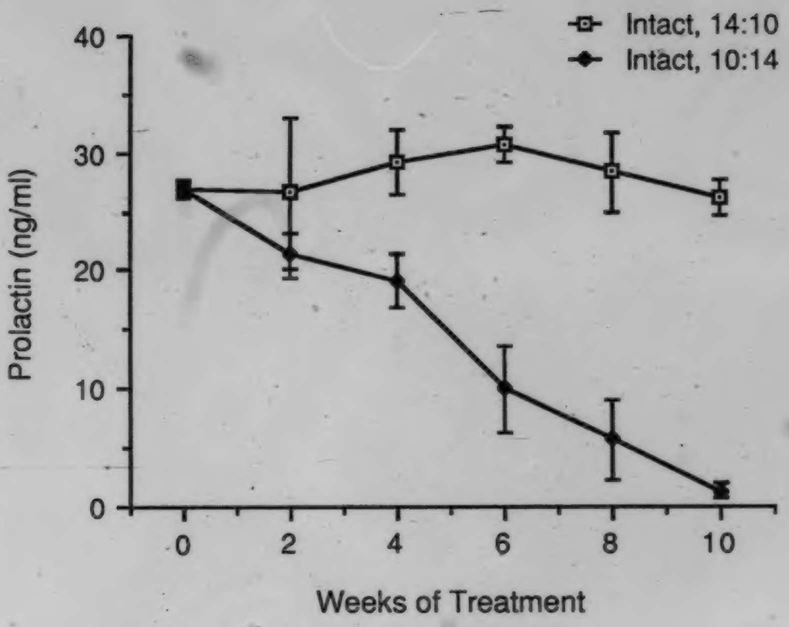

Fig. VII-19. Serum levels (mean ± SEM) of prolactin in hamsters that were sampled at day 0, and in intact hamsters maintained in long or short daily photoperiods.

PROLACTIN LEVELS

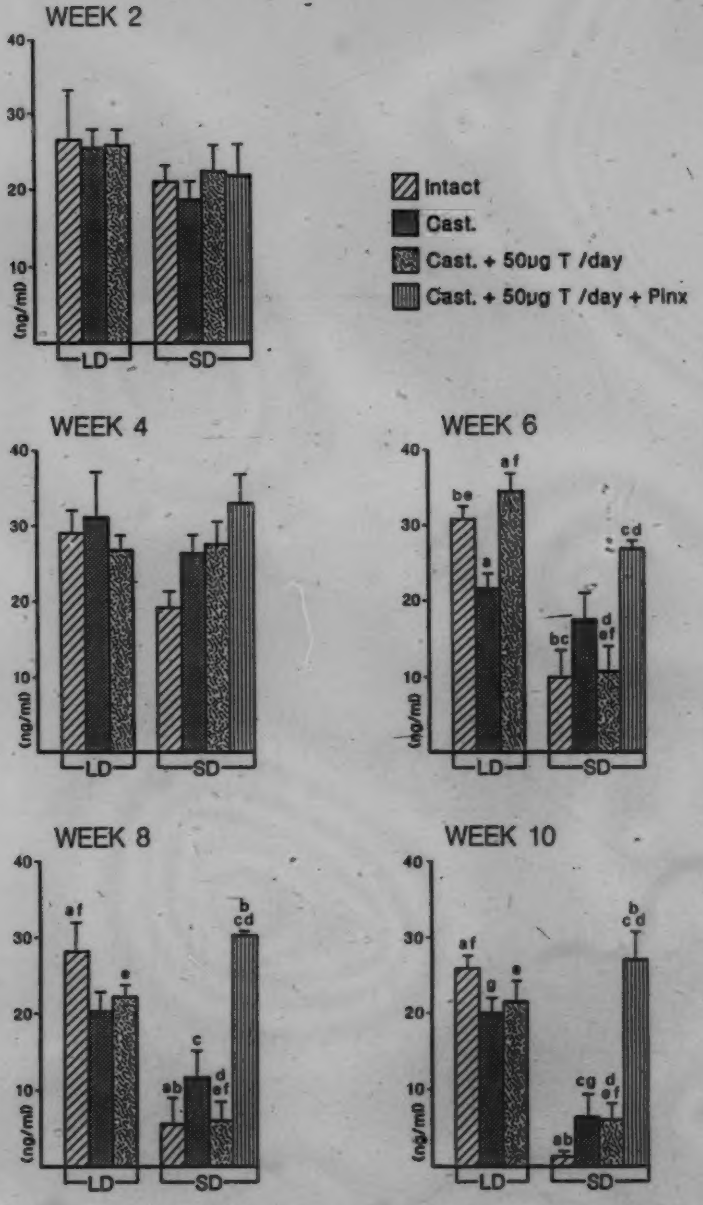

Fig. VII-20. Serum levels (mean ± SEM) of prolactin hormone in hamsters treated as described in Fig. VII-4. Groups with similar superscripts are signigicantly different from each other ($P < 0.05$).

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VIII- GENERAL DISCUSSION

Exposure of intact young adult male Syrian hamsters to short daily photoperiods consistently resulted in a marked reduction in the weights of the testes and accessory sex glands compared with animals kept in long daily photoperiods (Chapter II-VII). This supports and extends earlier reported studies that reduced weights of gonads, coagulating glands and seminal vesicles are the usual consequences of short-days exposure (Hoffman and Reiter, 1965; Gaston and Menaker, 1967; Reiter, 1975; Elliott, 1976; Pickard and Silverman, 1979; Steger et al., 1982, 1983). In all experiments presented in this study, among the accessory sex glands that were examined, the ventral lobes of the prostate lost less weight in response to short days than did other accessory sex glands. Such response of the ventral prostate to short days was also evident at the subcellular levels. For example, the fine structural changes in the secretory epithelial cells of the dorsal prostatic lobes in intact hamsters exposed to short days were more dramatic than those observed in the ventral lobes (Chapter IV).

In most of the secretory epithelial cells of the dorsal lobes of the prostate in hamsters maintained in short days, all indicators of cytological secretory activity were depressed or eliminated. The secretory cells of this gland which were normally characterized in animals maintained in long days by highly dilated cisternae of rough endoplasmic reticulum and the presence of apical bulges and blebs of various sorts, exhibited considerable regression of the cisternae of rough endoplasmic reticulum and an increase in the number of lysosomes when the animals were exposed to short days. In addition, at the luminal surface, microvilli, apical bulges and blebs were less pronounced in the secretory cells in short days than in long days. The secretory cells of the ventral prostate, on the other hand, which were characterized in animals maintained in long days by well developed rough endoplasmic reticulum with flattened cisternae, a large Golgi complex, membrane-bound secretory granules and the presence of microvilli at the luminal surface, were less influenced by exposure to short days. Although some of the secretory cells in short days showed signs of atrophy, most of the secretory cells were identical to those observed in long days. The most obvious changes in the atrophied cells were regression of the rough endoplasmic reticulum and Golgi complex and an increase in the number of electron-dense bodies.

In general, the fine structural study of the ventral and dorsal lobes of the prostate may denote that secretory activity associated with protein synthesis was suppressed in hamsters exposed to short days. This was confirmed by the biochemical assay of the total protein content in these glands (Chapter VII). Exposure of intact hamsters to short days resulted in a significant reduction in the total protein content of the ventral and dorsal lobes of the prostate, relative to protein values obtained from animals maintained in long days. The changes in the total protein content of the dorsal prostate in response to short days, were more dramatic than in the ventral prostate, which is consistent with the changes observed in the weight and fine structure of these glands. This indicates that the ventral prostate is less sensitive to short days than the dorsal prostate.

The differential response of the ventral and dorsal prostate to short days confirms our earlier observations reported by Buzzell et al. (1985) where light microscopic stereological techniques were used to examine the effects of short and long days on the structural integrity of the ventral and dorsal lobes of the prostate in intact hamsters. In that study, the ventral prostate reacted to short days with minor morphological changes relative to those observed in the dorsal prostate. However, in the present study, no noticeable changes were observed in the fine structure of the components of the interacinar tissue (i.e. connective tissue and smooth muscle fibers) in both lobes of the prostate, whereas in the study reported by Buzzell et al. (1985), the dorsal prostate of intact hamsters in short days showed a large increase in the volume fraction of the interacinar tissue, and there was an increase in the thicknesses of the lamina propria and muscular stroma, relative to those in long days.

Paralleling the involution of the testes and accessory sex glands, circulating levels of testosterone and prolactin were found to be consistently reduced in intact hamsters in short days, relative to levels seen in long days treated hamsters (Chapters II-VII). These results are in accord with observations reported by several other investigators (Berndtson and Desjardins, 1974; Pickard and Silverman, 1979; Matt and Stetson, 1980; Reiter, 1980; Borer et al., 1982).

Although several reports have indicated that atrophy of reproductive organs caused by exposure to short daily photoperiods is usually accompanied by decreases in circulating levels of LH and FSH (Reiter, 1973; Berndtson and Desjardins, 1974; Turek et al., 1975, 1976; Tamarkin et al., 1976; Goldman and Brown, 1979; Steger at al., 1984) the results obtained from the present study and those reported by other investigators (Reiter, 1975; Bex et al., 1978; Steger et al., 1985) suggest that a decline in peripheral levels of gonadotropins does not occur consistently in hamsters kept in short days.

The effects of short-day exposure on the weights and fine structure of the accessory sex glands and on reproductive hormone levels appears to be mediated by pineal secretion, as removal of the pineal gland negated the suppressive effects of short days (Chapter III, IV and VII). The present results confirm and extend published observations indicating that in hamsters that are pinealectomized, kept in darkness or blinded do not exhibit atrophy of the reproductive organs or decreases in the levels of reproductive hormones (Hoffman and Reiter; 1965; Reiter and Hester, 1966; Reiter, 1969; Demaine, et al., 1983; Jackson et al., 1984).

Although pinealectomy did not produce significant changes in the glandular epithelium of the ventral and dorsal prostate of hamsters maintained in short days, some of the secretory cells of the dorsal prostate showed larger Golgi complexes and more secretory granules than those normally seen in intact hamsters in long days (Chapter IV). This suggests an augmented activity of this gland by pinealectomy. The study reported by Lin and Wing (1978) also indicated that in blinded hamsters, the secretory cells of the seminal vesicles were extremely involuted, whereas pinealectomized or superior cervical ganglionectomized hamsters exhibited cytological features suggestive of enhanced activity.

Removal of the pineal gland from hamsters kept in long days had no significant effect on the weight of the reproductive organs, compared to sham operated animals kept in the same daily photoperiods (Chapter III). This is also in agreement with previous interpretations which suggest that hamsters are functionally or "physiologically pinealectomized" if maintained under long daily photoperiodic conditions; exposure to long days inhibits the pineal antagonistic effects on the reproductive system (Reiter, 1971, 1978). Therefore, surgical removal of the pineal gland from such animals has little or no effect on the neuroendocrine-gonadal axis.

The atrophy of the reproductive organs, fine structural changes observed in the secretory epithelial cells of the ventral and dorsal prostate and the marked decline in circulating levels of testosterone, simulate the changes reported for androgen deprivation, as by castration in hamsters and other rodent species (Ortiz, 1953; Dahl and Kjaerheim, 1973; Dahl and Tveter, 1973; Buzzell et al., 1985). Castration in long and short days resulted in a significant reduction in the weights of the accessory sex glands and in a marked drop in circulating testosterone levels (Chapter V and VII). The fine structural changes observed in the secretory cells of the ventral and dorsal lobes of the prostate in hamsters exposed to short days were generally similar to those reported in fine structural studies of the post-castration involution of the accessory sex glands in rodents, such as the ventral, lateral and dorsal lobes of the prostate gland in rat (Brandes, 1966; Brandes and Groth, 1963; Dahl and Kjaerheim, 1973; Dahl and Tveter, 1973) and the coagulating glands of rats and hamsters (Brandes et al., 1962; Dahl and Tveter, 1973; Cavazos, 1975). This suggests that the observed changes in weight and morphology of the accessory sex glands are due to a decrease in circulating levels of testosterone.

Although prolactin has been implicated as an important hormone in reproduction, the results from the present study indicate that the presence of excess prolactin (secreted by pituitary glands grafted under the kidney capsule) did not prevent the atrophic response of the accessory sex glands to short days, except for the weight of the seminal vesicles which did not differ significantly from similarly treated animals maintained in long days (Chapter VI). The differential response of the accessory sex glands to excess prolactin might be related to differences in prolactin dependence among these organs. Although prolactin binding sites have been demonstrated in the ventral, lateral and dorsal lobes of the prostate and seminal vesicles of rats (Aragona and Friesen, 1975; Barkey et al., 1977, 1979; Witorsch, 1978, 1981; Thompson et al., 1982), the literature reveals many conflicting reports concerning the degree of responsiveness of the accessory sex glands in intact and castrated animals to circulating prolactin.

Although prolactin reportedly potentiates the effects of LH on the biosynthesis of testicular androgens (Hafiez et al., 1972), the present data indicate that the presence of excess prolactin does not prevent the suppressive effects of short days on circulating levels of testosterone, even though peripheral levels of LH were similar to those observed in long days (Chapter VI). These results may imply that the decrease in circulating prolactin levels associated with short-day exposure is not the major factor resulting in the atrophy of the reproductive organs or in decreasing testicular output of testosterone. Accordingly, an experiment was conducted to examine whether the atrophy of the acessory sex glands in short days was primarily due to the reduction in peripheral levels of testosterone, or if other factors are involved.

The effect of short days on the accessory sex glands was examined in the presence of levels of testosterone capable of maintaining the normal size of the accessory sex glands in castrated hamsters. Furthermore, an attempt was made to reveal the temporal pattern of changes in the weights and functional activities of the accessory sex glands, and in serum reproductive hormone levels throughout the ten weeks of exposure to short days (Chapter VII).

Exposure of intact hamsters to short days resulted in a gradual decline in the weights of the testes and accessory sex glands, and the results indicate that 6-8 weeks was required to induce a significant reduction in the weights of these organs. These results are consist with results described in several previous reports (Reiter, 1968; Berndtson and Desjardins, 1974; Bartke et al., 1981; Steger et al., 1982; Jackson et al., 1984).

The decrease in weights of the ventral and dorsal lobes of the prostate was accompanied by a decline in total protein content of these glands. Short days also resulted in a marked reduction in the DNA content of the dorsal prostate, and a nonsignificant decline in the ventral prostate. The decrease in the weight and protein content of these glands was more rapid than the decrease in the DNA content. Castration in long or short days also resulted in a significant reduction in the weight, DNA and protein contents of these glands. The reduction in the weight, DNA and protein content of the dorsal prostate with both treatments (short-day exposure, and castration) was more severe than in the ventral prostate, confirming the earlier observations presented in this study, and those reported by Ortiz (1953), Schmidt and Visek (1977), and Buzzell et al., (1985), indicating that the ventral prostate shows less reponse to short days and to castration than do other accessory sex glands.

Although short days and castration resulted in a significant reduction in the weight, DNA and protein content of the accessory sex glands, there were temporal differences in the initiation of these responses. The rate of decline of DNA and protein content and the decrease in the weight of the accessory sex glands with short days were slower than with castration.

A second point of interest from this investigation is the fact that, although circulating prolactin levels were significantly reduced in short days (intact, castrated, testosterone-treated castrates), the presence of an adequate supply of testosterone in castrated hamsters prevented the suppressive effects of short days on the accessory sex glands. All parameters measured (weight, DNA and protein content) with respect to the accessory sex glands in castrated hamsters that received testosterone and were maintained in short days were comparable to those subjected to a similar treatment but kept in long days. This suggests that the atrophy of the accessory sex glands in short days is primarily due to a significant reduction in circulating testosterone levels.

The fall in the total amount of DNA and protein after exposure of intact hamsters to short days and in castrated hamsters in long or short daily photoperiods may reflect a decline in the total number of cells due to cell death (Coffey et al., 1968; Barham et al., 1980; Okamoto et al., 1982; Isaacs, 1984). This may have resulted from the significant drop in serum testosterone levels with both treatments. Testosterone has been reported to have the agonistic ability to stimulate the proliferation of prostatic cells, and also to have antagonistic ability of inhibiting prostatic cell death (Isaacs, 1984). Therefore, the involution of the prostate following exposure to short days or after castration could be due to decreased antagonistic effects of androgen on prostatic cell death.

Another finding in the present study is that serum LH and FSH levels in testosterone-treated castrates in short days were consistently lower than those in the corresponding group in long days (Chapter VII). This reduction in serum levels of gonadotropins appears to be dependent on the presence of the pineal gland, as removal of the pineal gland in testosterone-treated castrates prevented the suppressive effects of short days on serum gonadotropins. The low levels of serum gonadotropins observed in testosterone-treated castrates in short days might be due to an increase in the sensitivity of the hypothalamic-pituitary axis to the negative feedback effects of testosterone (Tamarkin et al., 1976; Turek, 1977). The increase in the sensitivity of the hypothalamic-pituitary axis to testosterone might be mediated by the pineal hormone melatonin. Sisk and Turek (1982) indicated that daily melatonin injections given to testosterone-treated castrated hamsters in long days increased the negative feedback effects of testosterone on gonadotropin release.

It appears from the results presented in this study that the effects of short photoperiods on the reproductive system are primarily mediated by the pineal gland, since these changes do not occur in the absence of this gland. Since the biosynthesis of melatonin in the pineal gland is controlled by the lighting schedule, this indole may be the pineal hormone that modulates the status of the reproductive system via its action on the neuroendocrine-gonadal axis.

It can be concluded from the present results that: 1) exposure of intact adult Syrian hamsters to short photoperiods results in a significant reduction in the weight, DNA and protein contents and marked changes in the fine structure of the accesory sex glands; 2) the reduction in the weight, DNA and protein content of the accessory sex glands is more rapid with castration than with exposure to short days; 3) short-day exposure results in a consistent reduction in the circulating levels of testosterone and prolactin; 4) the effects of short days on the accessory sex glands are primarily due to a decrease in circulating testosterone levels, and this could be mediated via the effects of the photoperiod on the neuroendocrine-gonadal axis; and 6) the suppressive effects of short days on the reproductive organs and on circulating reproductive hormones are pineal mediated phenomena, as removal of the pineal gland negates the effects of short days.

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APPENDIX 1
Light and Electron Microscopy

Sodium Phosphate Buffer, 0.13 M, pH 7.4

Monobasic sodium phosphate ($NaH_2PO_4.H_2O$)	0.34 g
Dibasic sodium phosphate ($Na_2HPO_4.7H_2O$)	2.82 g
Deionized water	100 ml

Fixative

3% glutaraldehyde, 3% paraformaldehyde in 0.13 M sodium phosphate buffer pH 7.4

phosphate buffer 0.13 M pH 7.4	45 ml
paraformaldehyde	1.5 g

heated in Erlenmeyer flask to about 70 °C, mixed on magnetic stirrer until suspension was clear

glutaraldehyde 25%	6 ml

Lee's Methylene Blue-Basic Fuchsin

Methylene blue stock:

Methylene blue	0.13 g
Deionized water	100 ml

Basic fuchsin stock:

Basic fuchsin	0.13 g
Deionized water	100 ml

Sodium phosphate buffer, 0.2 M, pH 7.4, stock:

Monobasic sodium phosphate ($NaH_2PO_4.H_2O$)	0.26 g
Dibasic sodium phosphate ($Na_2HPO_4.7H_2O$)	2.17 g
Deionized water	50 ml

Staining solution mixture:

Methylene blue solution	12 ml
Basic fuchsin solution	12 ml
Phosphate buffer, 0.2 M	21 ml
Ethanol (95%)	15 ml

Filter

Toluidine Blue, 1%

Deionized water	50 ml
Sodium borate	0.5 g

Toluidine blue	0.5 g

Filter

Reynolds Lead Citrate

Deionized water	30 ml
Lead nitrate	1.33 g
Sodium citrate, monohydrate	1.76 g

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prepared in 50 ml Erlenmyer flask, shaked at intervals for 30 minutes.

Sodium hydroxide, 1 N	8 ml

Mixed to dissolve lead completely.

Deionized water	12 ml

Embedding Media

Glycol methacrylate medium (JB-4 embedding kit):

Infiltration medium (catalyzed solution A):

Solution A	100 ml
catalyst	0.9 g

Mixed with a magnetic stirrer at room temperature until the catalyst was completely dissolved.

Embedding medium:

Catalyzed solution A	25 parts
Solution B	1 part

Araldite embedding medium:

Araldite 502	30.0 ml	- 33.3 g
DDSA	23.4 ml	- 23.4 g
DMP-30	0.8 ml	- 0.8 g

Mixed

Tissue Processing for Light Microscopy

Fixation:

3% glutaraldehyde, 3% paraformaldehyde in 0.13 M sodium phosphate buffer, pH 7.4	overnight at 4 °C

Rinse:

Sodium phosphate buffer, 0.13 M, pH 7.4	3 x 10 min

Dehydration:

50% ethanol	4-6 hr
70% ethanol	overnight
95% ethanol	2 x 1 hr

Infiltration:

95% ethanol + Catalyzed solution A, 3:1	30 min
95% ethanol + catalyzed solution A, 1:1	30 min
95% ethanol + catalyzed solution A, 1:3	1 hr
Catalyzed solution A	2 x 1.5 hr

Embedding and polymerization:

JB-4 embedding medium	overnight, room temp.

Tissue Processing for Electron Microscopy

Fixation:

3% glutaraldehyde, 3% paraformaldehyde in 0.13 M sodium phosphate buffer, pH 7.4	overnight at 4 °C

Rinse:

Sodium phosphate buffer, 0.13 M, pH 7.4	3 x 10 min

Postfixation:

1% Osmium tetroxide in 0.13 M sodium phosphate buffer, pH 7.4	2 hr

Dehydration:

50% Ethanol	10 min
70% Ethanol	10 min
80% Ethanol	10 min
Staining *en block*, 2% uranyl acetate in 80% ethanol	15 min
90% Ethanol	10 min
100% Ethanol	3 x 10 min
100% Propylene oxide	3 x 10 min

Infiltration:

Propylene oxide + Araldite mixture 1:1	1 hr
propylene oxide + Araldite mixture 1:3	1 hr
Araldite mixture	overnight

Embedding:

Araldite mixture (fresh resin).

Polymerization:

At 45 °C	12 hr
At 70 °C	24 hr

APPENDIX 2
DNA and Protein Assay

Stock Buffer

Trizma Base, 1 M, pH 8 at 22 °C
(121.1 gm per 1L deionized water)

Trizma Base, 2 M, pH 7.4 at 22 °C
(242.2 gm per 1L deionized water)

EDTA (disodium salt), 0.15 M
(55.8 gm per 1L deionized water)

EDTA (disodium salt), 0.2 M
(74.448 gm per 1L deionized water)

Working Buffers

Tris-EDTA-Glycerol (TEG), pH 7.5 at 22 °C

Trizma Base (2 M)	0.5 ml
EDTA (0.15 M)	1 ml
Glycerol	10 ml
Deionized water	88.5 ml

After mixing the above stock buffer solutions, the final concentration of the TEG buffer was:

10 mM trizma Base
1.5 mM EDTA
10% (v/v) Glycerol

DNA Assay Working Buffer
(Tris-EDTA-Ethidium bromide, pH 8 at 22 °C)

Trizma Base (1 M)	5 ml
EDTA (0.2 M)	2.5 ml
Ethidium Bromide (4 mg per 1 ml of H_2O)	125 µl

*Deionized water to make 1L

After mixing the above solutions, the final concentration of the buffer was:

5 mM Trizma Base
0.5 mM EDTA
0.5 µg/ml Ethidium Bromide

Stock Solutions for Protein Assay

Copper-tartrate-carbonate (CTC) reagent:

0.1% (w/v) $CuSO_4 \cdot 5 H_2O$
0.2% (w/v) Sodium Potassium Tartrate
10% (w/v) Na_2CO_3

The Na_2CO_3 was dissolved in about one-half the final volume and added slowly to a solution of copper sulfate-tartrate (also about one-half volume) while stirring. The solution was kept refrigerated.

Sodium dodecyl sulfate (SDS), 5% (w/v).
NaOH, 0.8 M
Folin-Ciocalteu phenol reagent, 2N
Bovine serum albumin (BSA) standard, 1 mg/ml

Working Solutions

Reagent A:

This solution was prepared every three or four days by mixing the following stock solutions:

CTC reagent ....one part
5% SDS ....two parts
NaOH 0.8 M ....one part

Reagent B:

Folin-Ciocalteu phenol reagent, 2 N ....one part
deionized water ....five parts

Working Standard Solutions

BSA 1 mg/ml (ml)	Deionized H_2O (ml)	Final Conc. mg/ml
0.000	0.500	0.000
0.025	0.475	0.050
0.050	0.450	0.100
0.100	0.400	0.200
0.150	0.350	0.300
0.200	0.300	0.400
0.250	0.250	0.500
0.300	0.200	0.600
0.350	0.150	0.700